HISTOLOGY

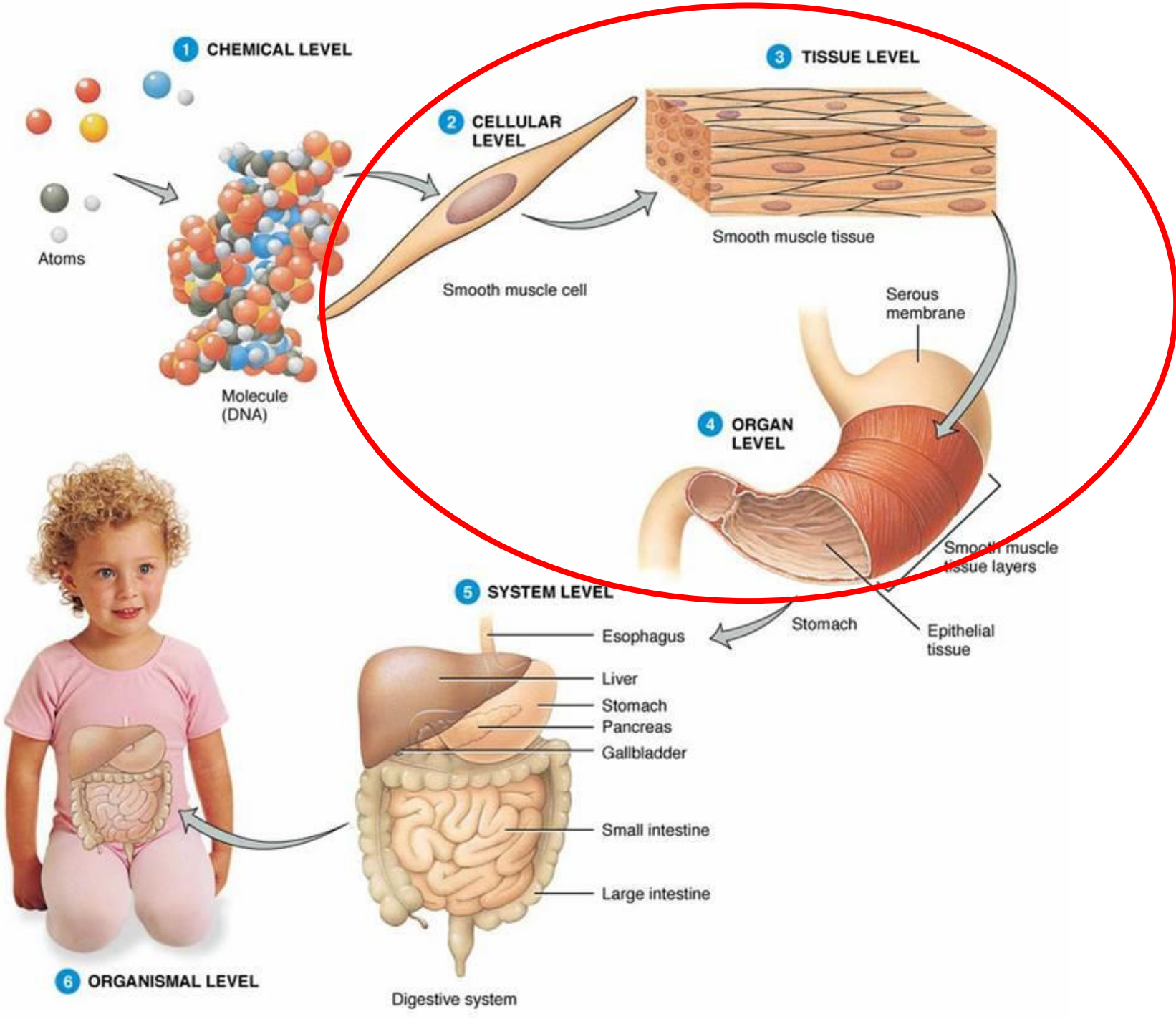
Scientific discipline studying structure of cells and tissues of multicellular organisms

1/ cytology

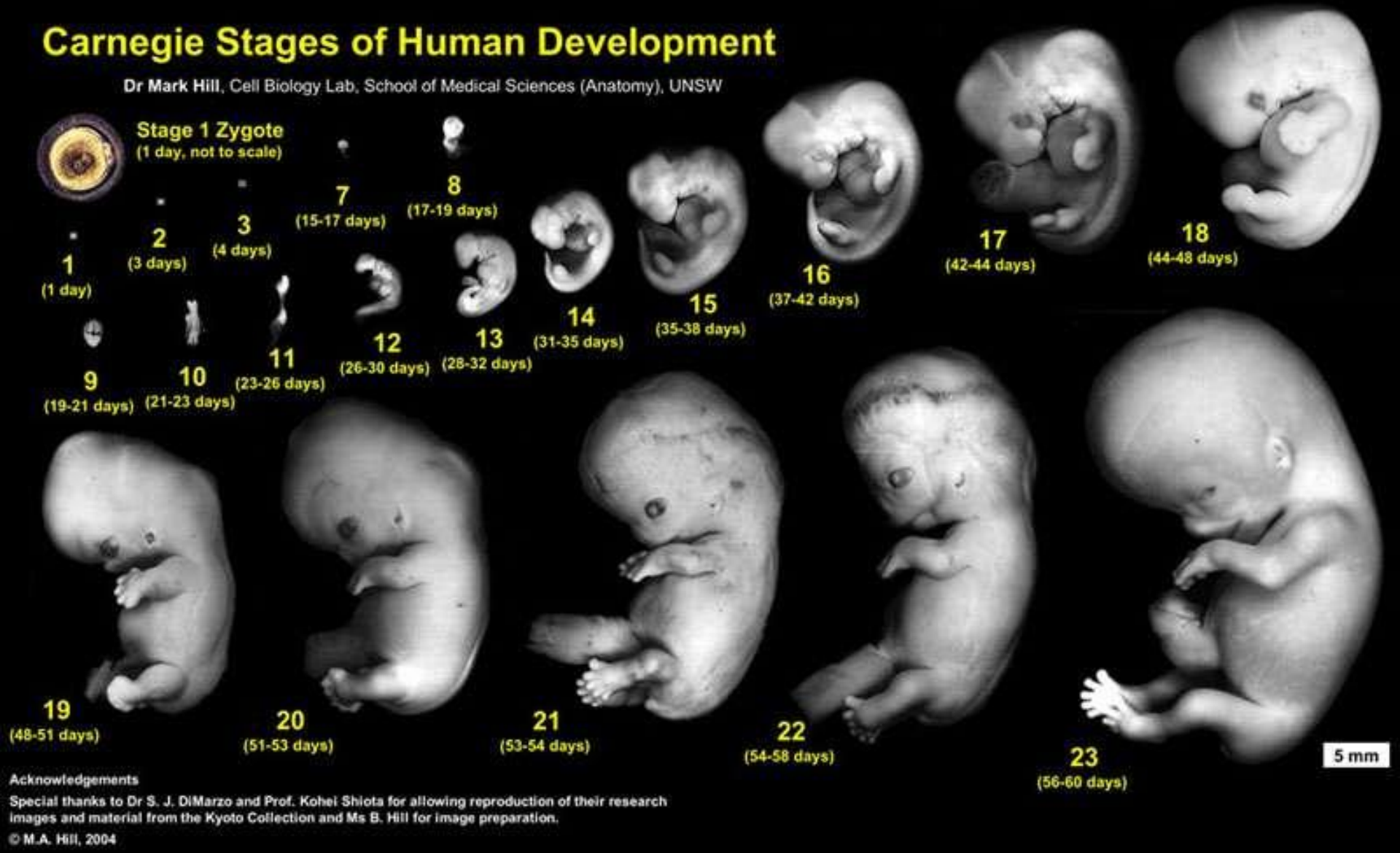
2/ general histology

3/ microscopic anatomy

MUDr. Jiří Uhlík, Ph.D.
jiri.uhlik@lfmotol.cuni.cz

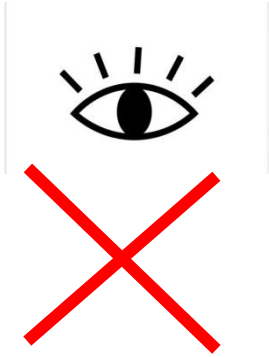

1 CHEMICAL LEVEL
Atoms
Molecule (DNA)
2 CELLULAR LEVEL
Smooth muscle cell
3 TISSUE LEVEL
Smooth muscle tissue
4 ORGAN LEVEL
Serous membrane
Smooth muscle tissue layers
Epithelial tissue
Stomach
5 SYSTEM LEVEL
Esophagus
Liver
Stomach
Pancreas
Gallbladder
Small intestine
Large intestine
Digestive system
6 ORGANISMAL LEVEL

EMBRYOLOGY

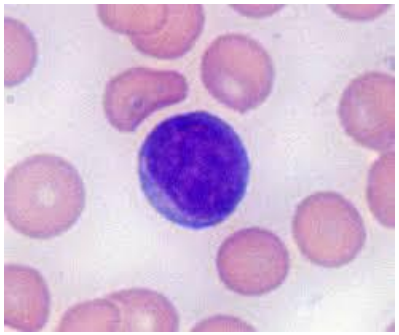


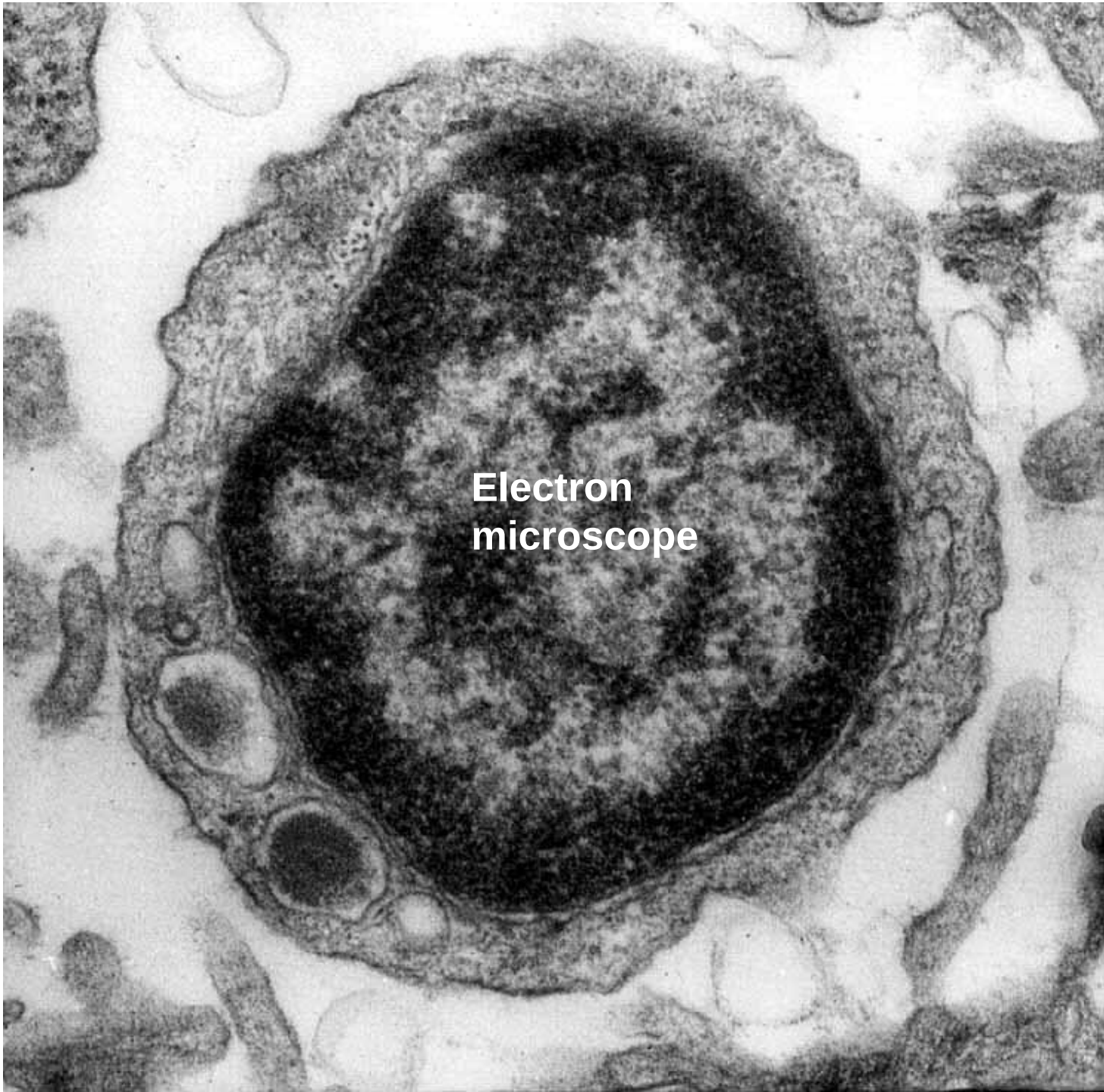
Acknowledgements

Special thanks to Dr S. J. DiMarzo and Prof. Kohei Shiota for allowing reproduction of their research images and material from the Kyoto Collection and Ms B. Hill for image preparation.



Light microscope





Largest cells 150 µm

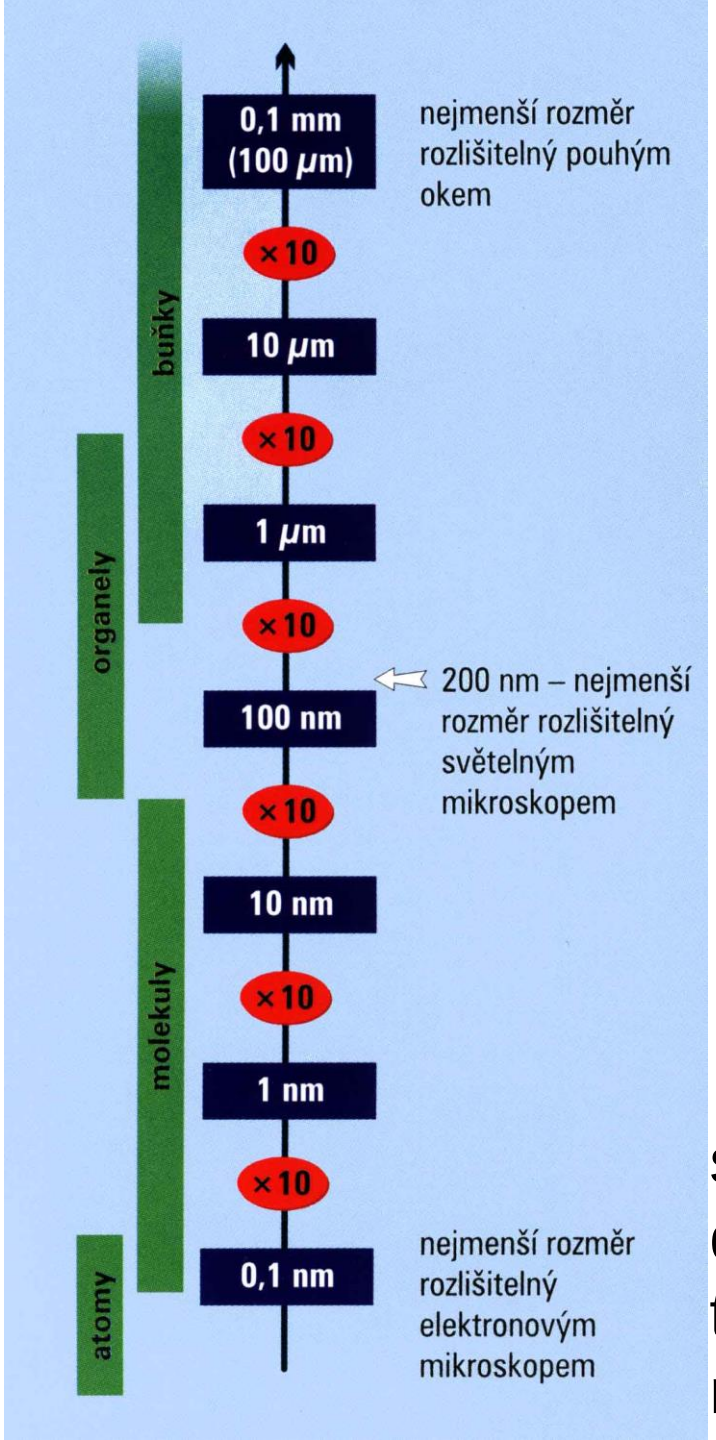
Average human cells 10 – 20 µm

Largest organelles 1 – 2 µm

Ribosomes 20x30 nm

Cytoskeleton 5 – 24 nm

Membrane 7,5 nm



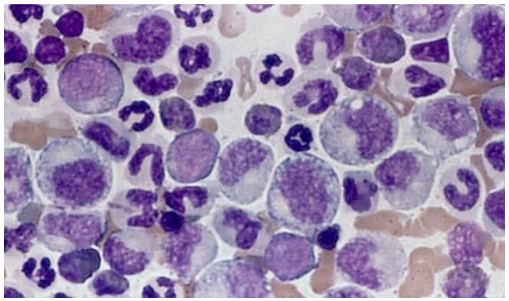
smallest dimension distinguishable by the naked eye

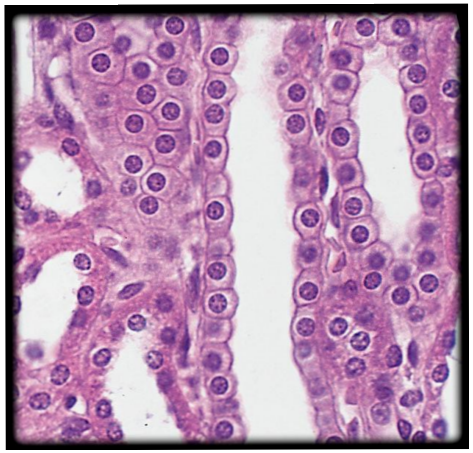
200 nm - smallest dimension distinguishable by the light microscope

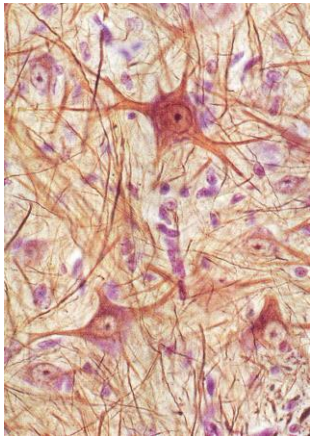
smallest dimension distinguishable by the electron microscope

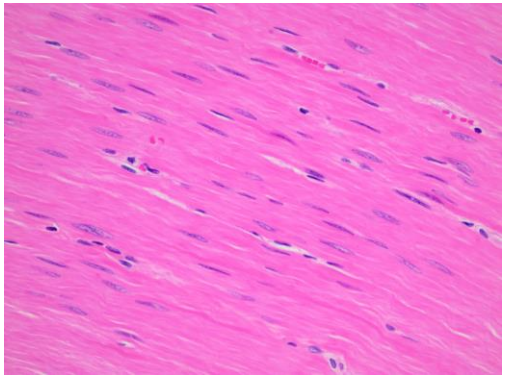
CELL

basic structural and functional unit of a living matter





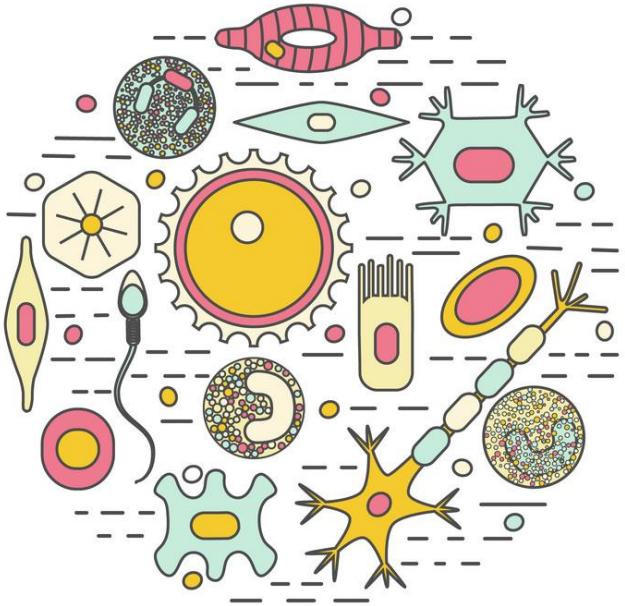




Number of cells in human body

30,000,000,000,000

cca 200 types of cells



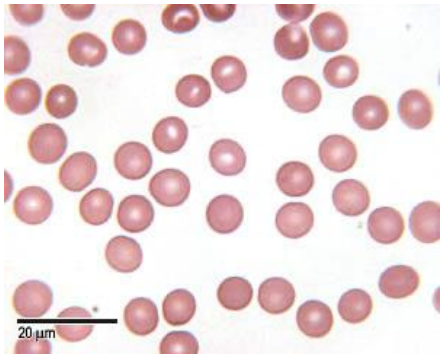
Size of cells

average human cell
10-20 μm

largest – oocyte 150 μm

smallest – chief cells of
parathyroid glands
or small granular neurons
of cerebellum
4-5 μm

standard
erythrocyte
7.5 μm



COMPONENTS OF EUCARYOTIC CELL

protoplasm = karyoplasm + cytoplasm

karyoplasm = content of nucleus

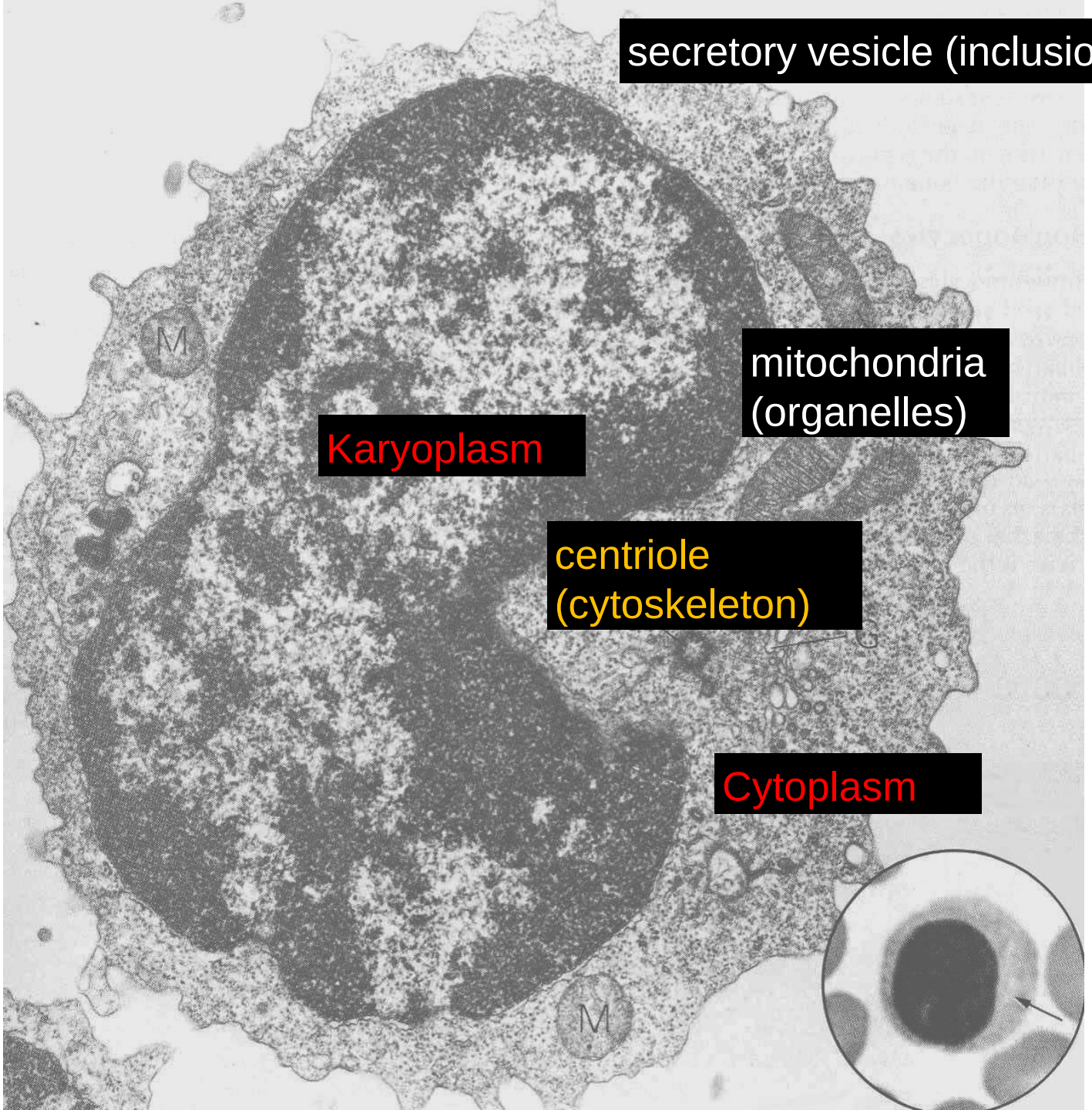
cytoplasm = matrix (cytosol) + cytoplasmic structures

1/ organelles – membranous

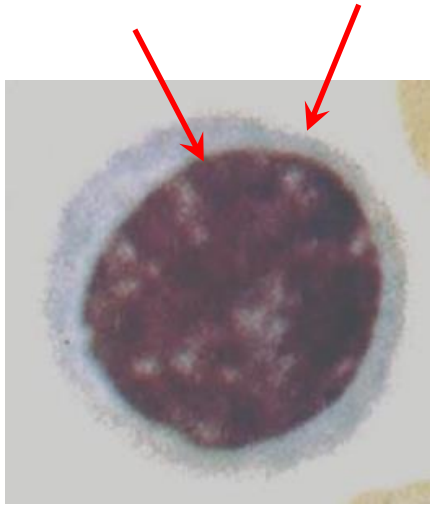
– non-membranous

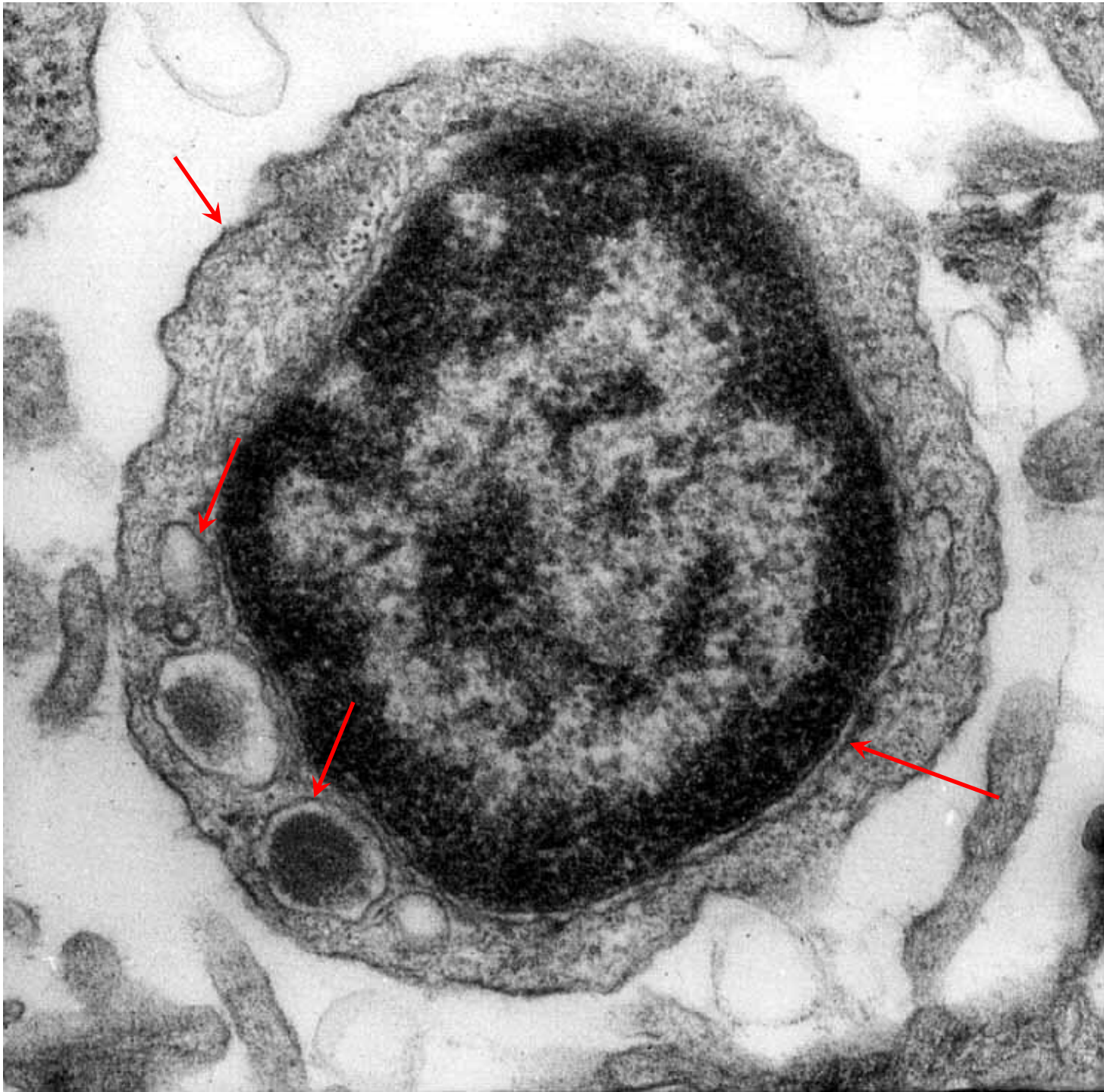
2/ cytoplasmic inclusions

3/ elements of cytoskeleton


secretory vesicle (inclusion)
mitochondria (organelles)
Karyoplasm
centriole (cytoskeleton)
Cytoplasm
M
M

Membrane





Membranous organelles

permanently present in a cell
enveloped by a membrane
containing enzymes for proper needs of a cell

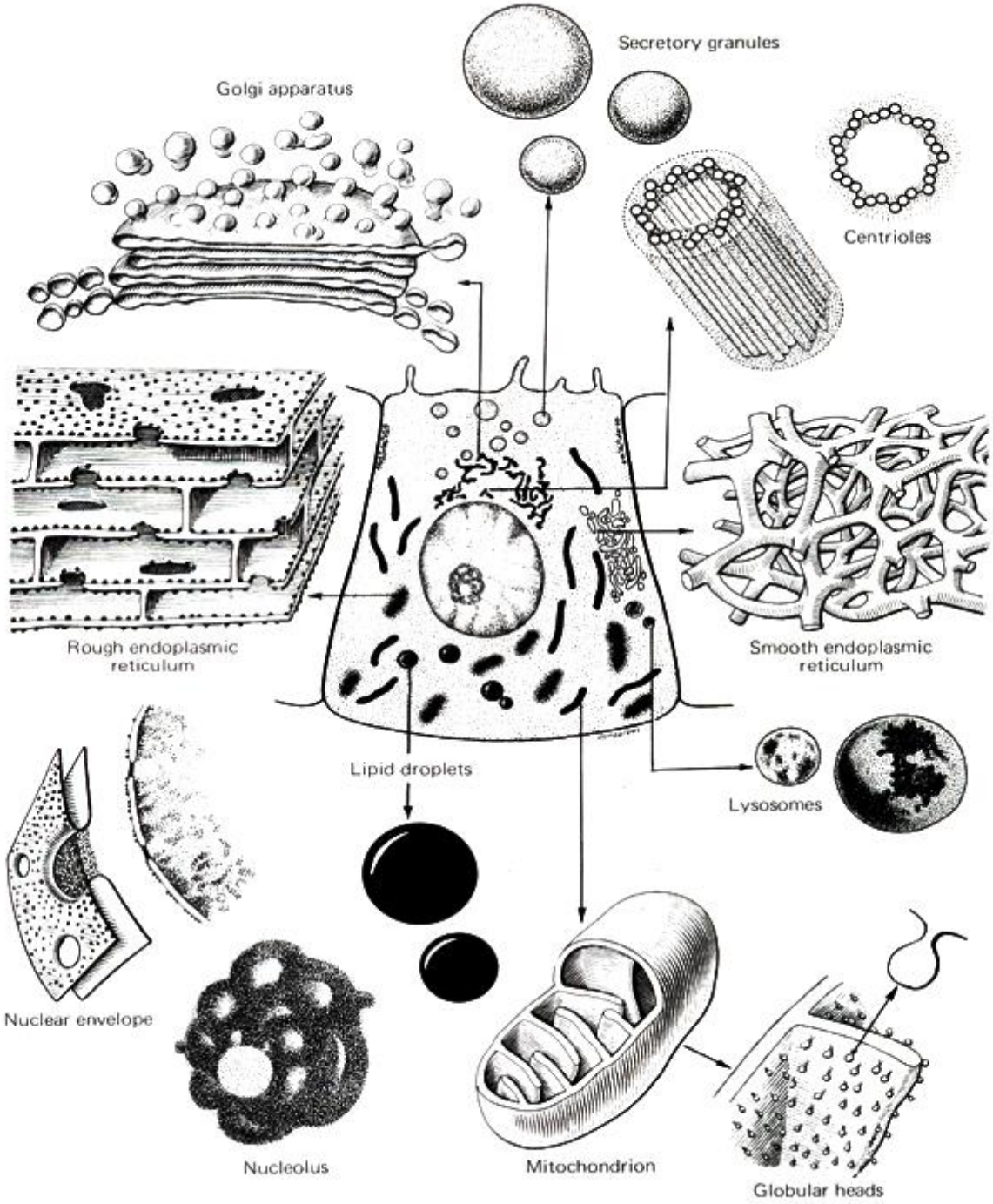
mitochondria

endoplasmic reticulum (smooth and rough)

Golgi complex

lysosomes

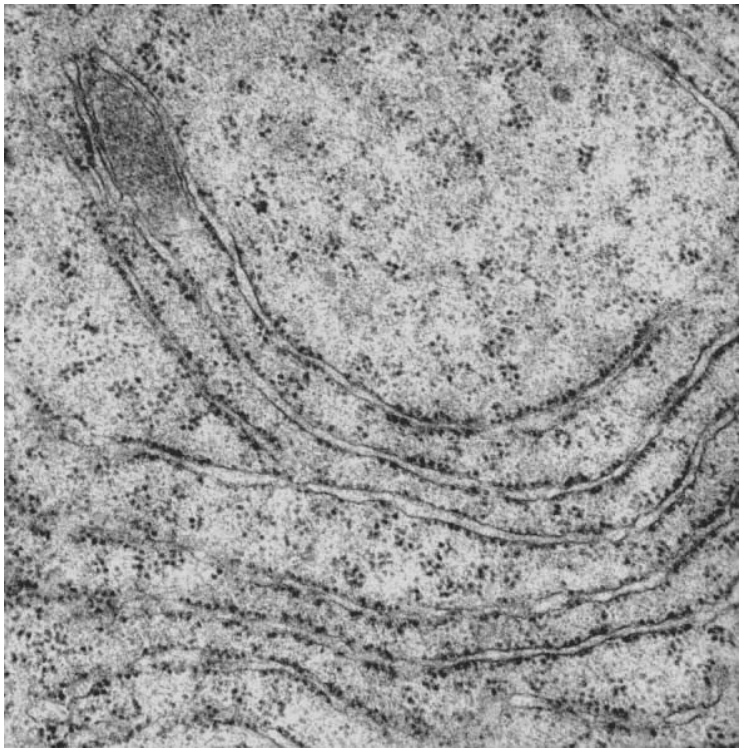
peroxisomes


Secretory granules
Golgi apparatus
Centrioles
Rough endoplasmic reticulum
Smooth endoplasmic reticulum
Lipid droplets
Lysosomes
Nuclear envelope
Nucleolus
Mitochondrion
Globular heads

Non-membranous organelles

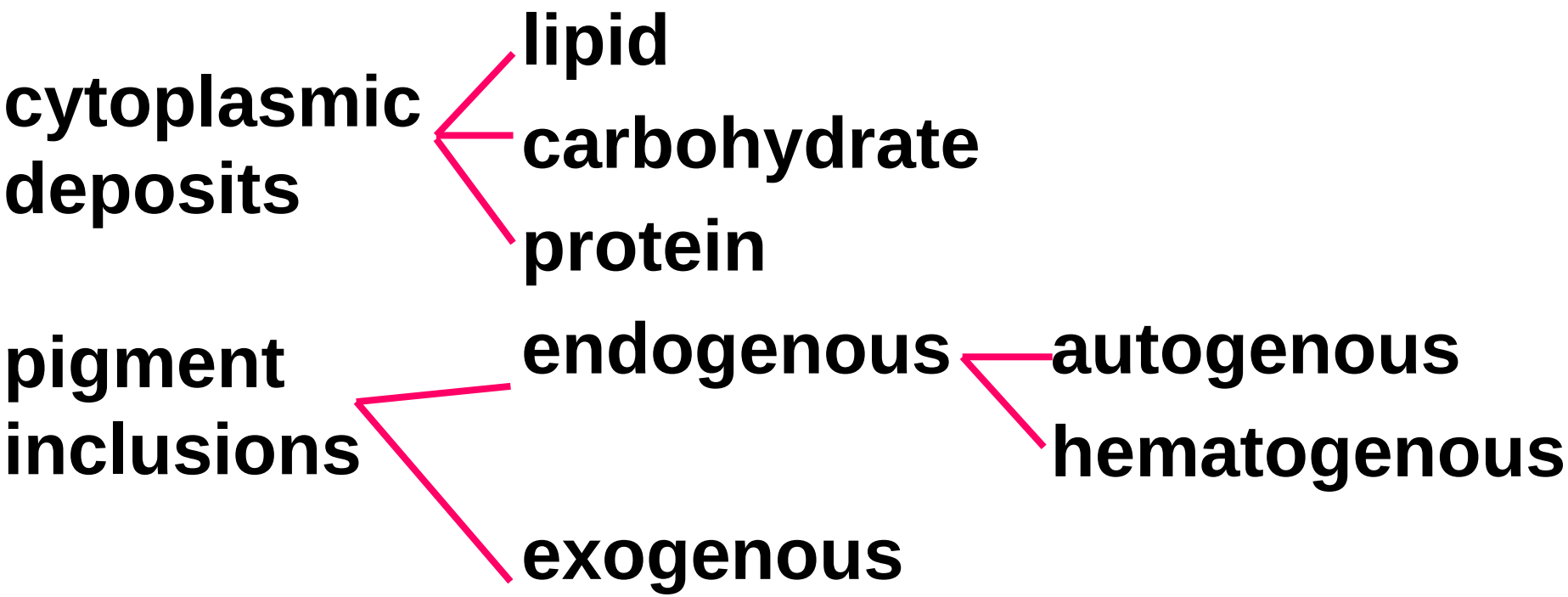
permanently present in a cell
NOT enveloped by a membrane
containing enzymes for proper needs of a cell

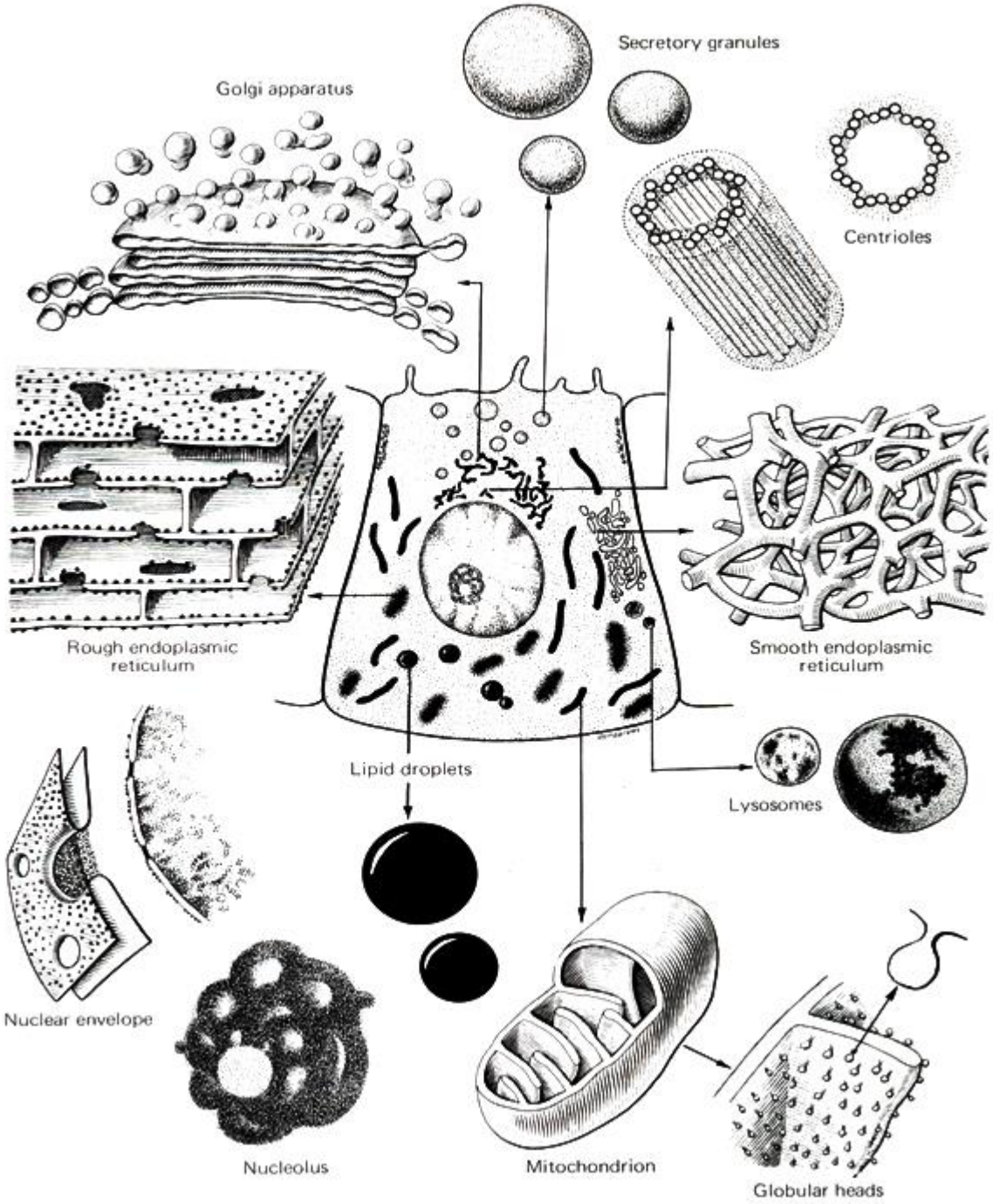
- **ribosomes**
- **proteasomes**
- **chaperones**
- **vaults (nanocapsules)**



Cytoplasmic inclusions

need not be in a cell at all, or transitory, or develop gradually
enveloping membrane may be present
if enzymes contained, they are not engaged in the cell metabolism

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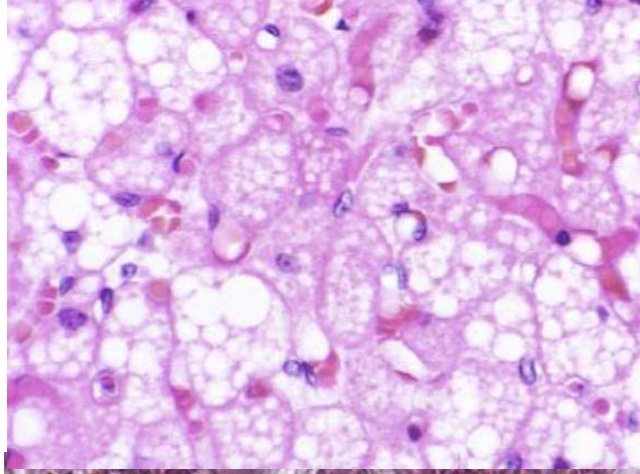
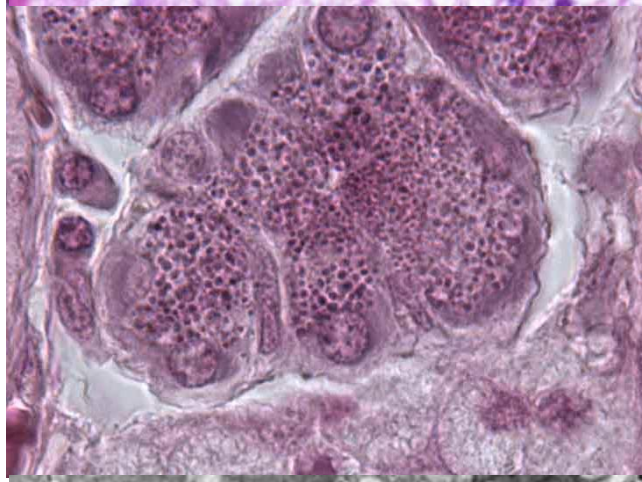
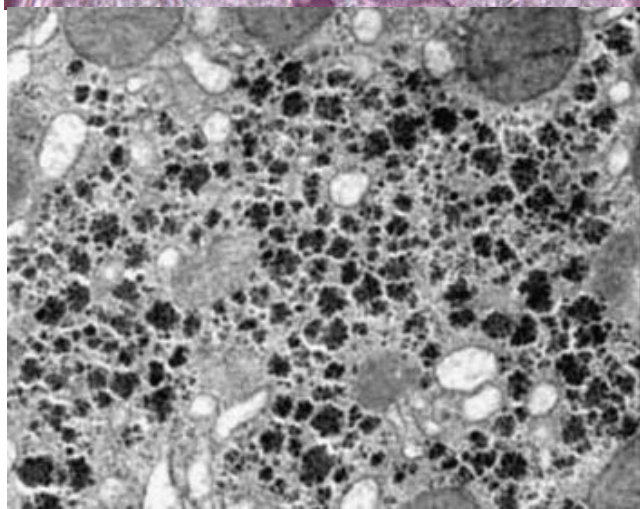






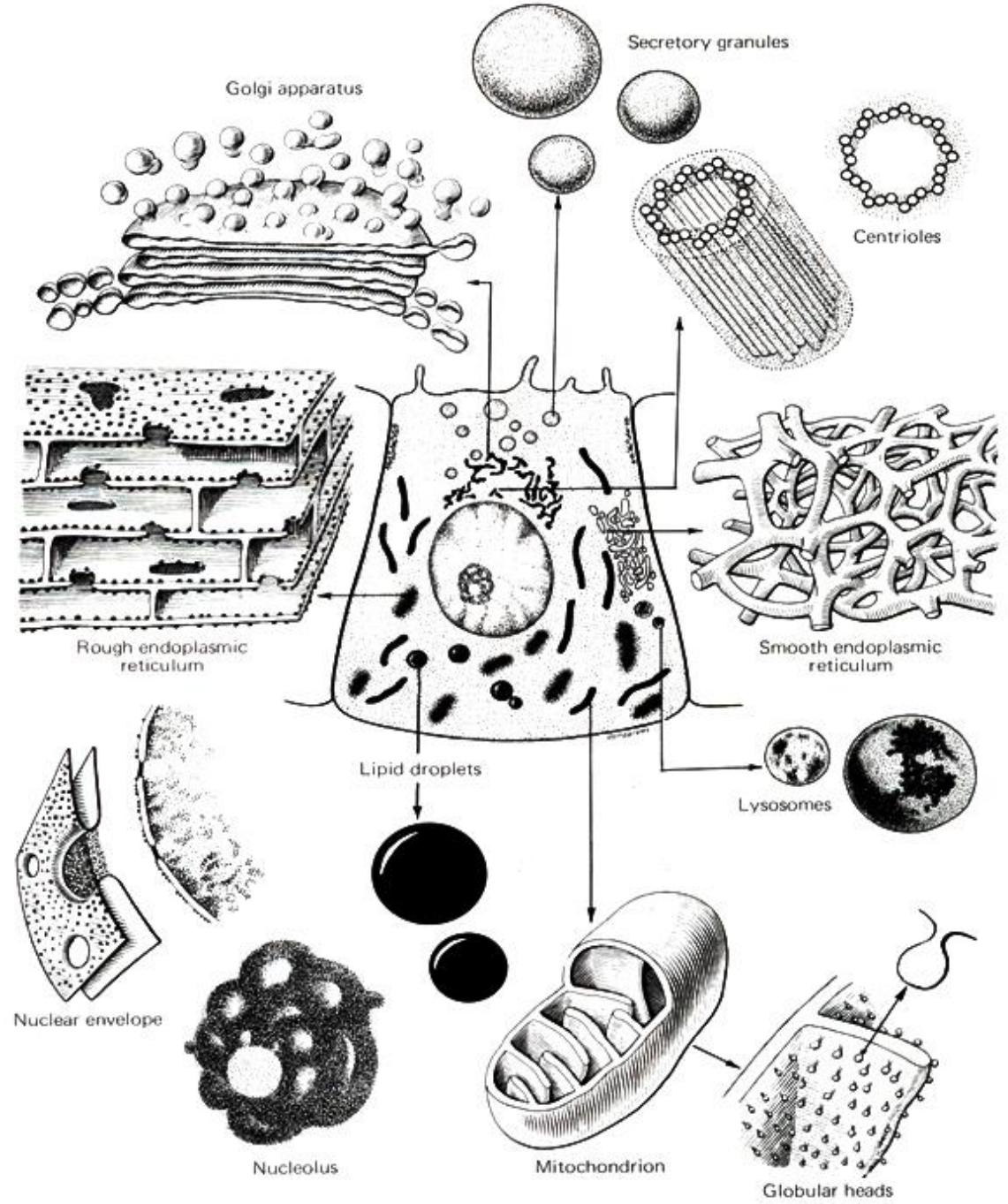
Cytoskeleton

chains of protein molecules
NOT enveloped by membrane
usually very labile and dynamic

microfilaments (5-7 nm)

intermediate filaments (10-12 nm)

microtubules (24 nm)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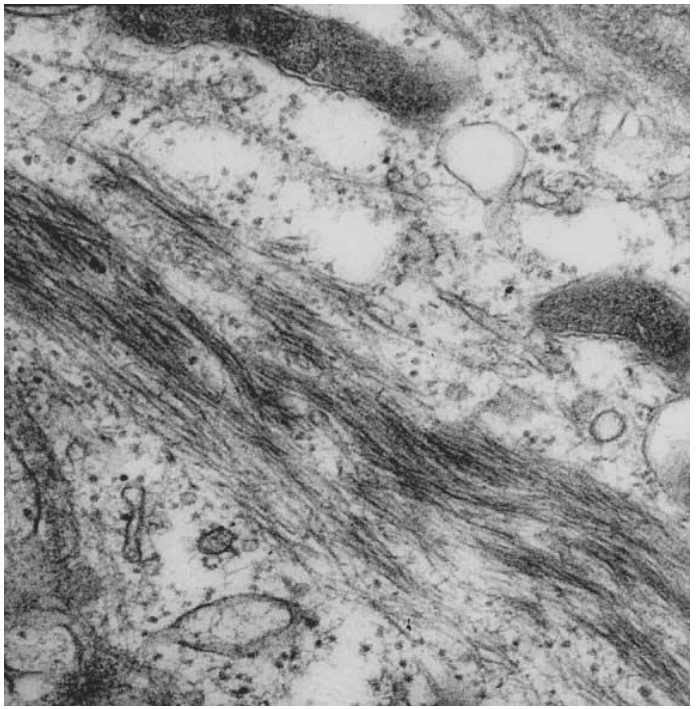
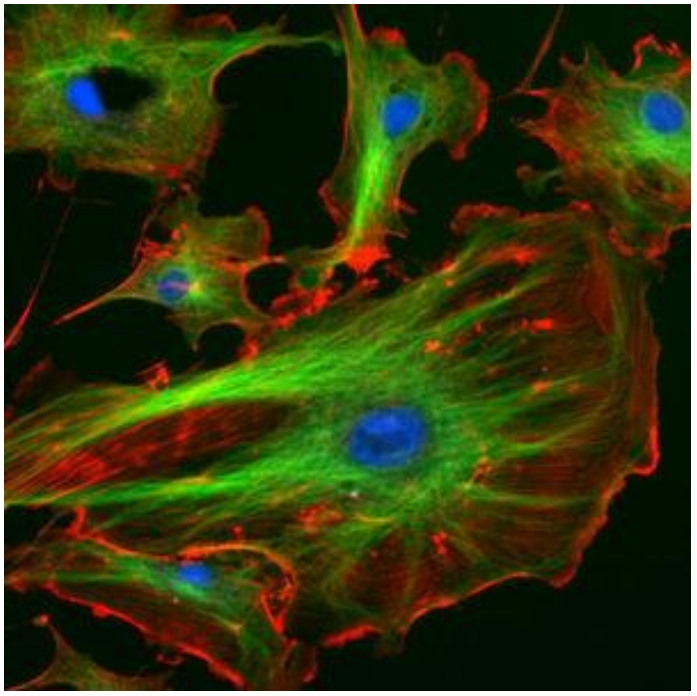
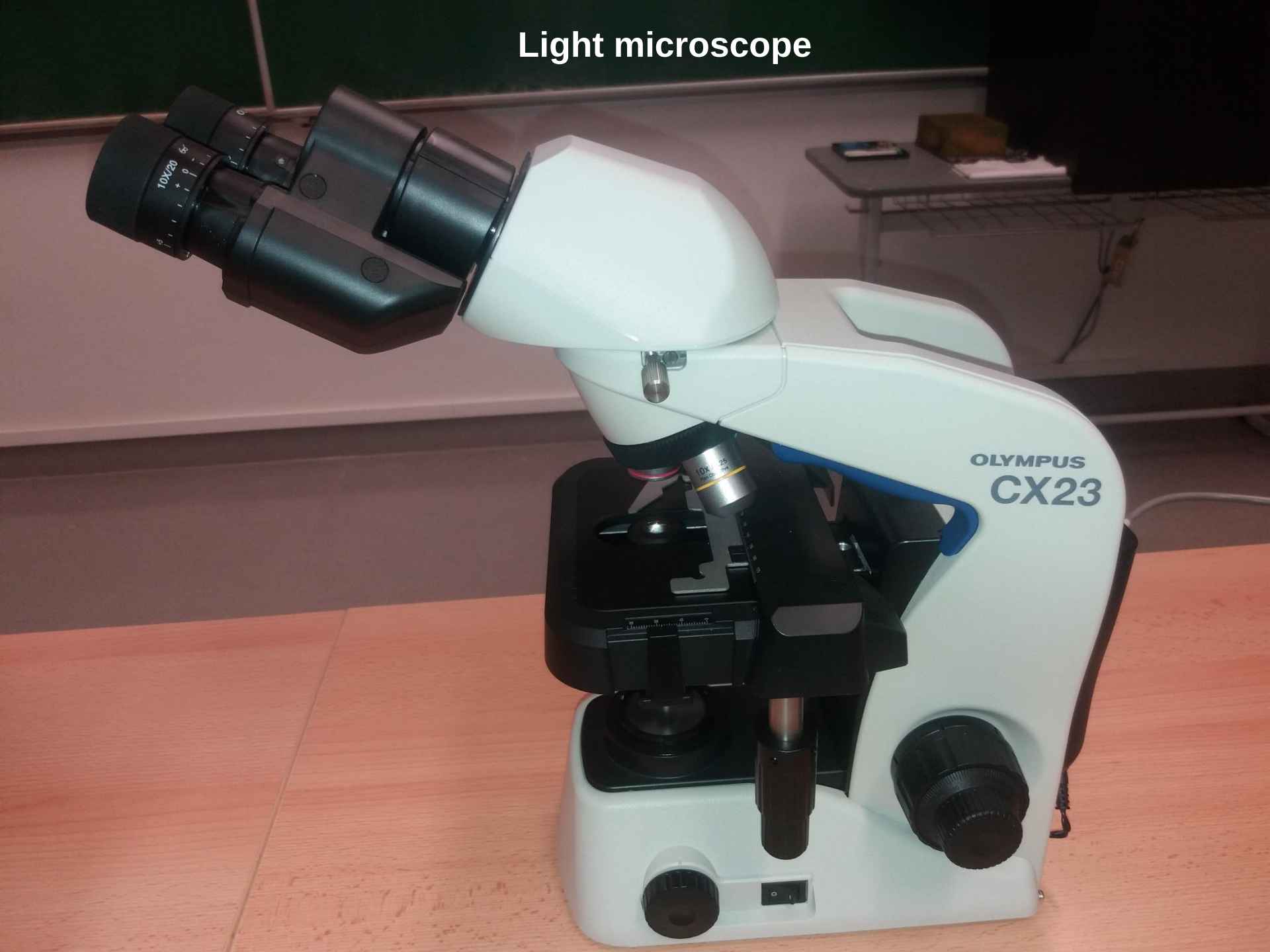




BASIC HISTOLOGICAL TECHNIQUES

Light microscope



Transmission electron microscope (TEM)

LIGHT MICROSCOPY

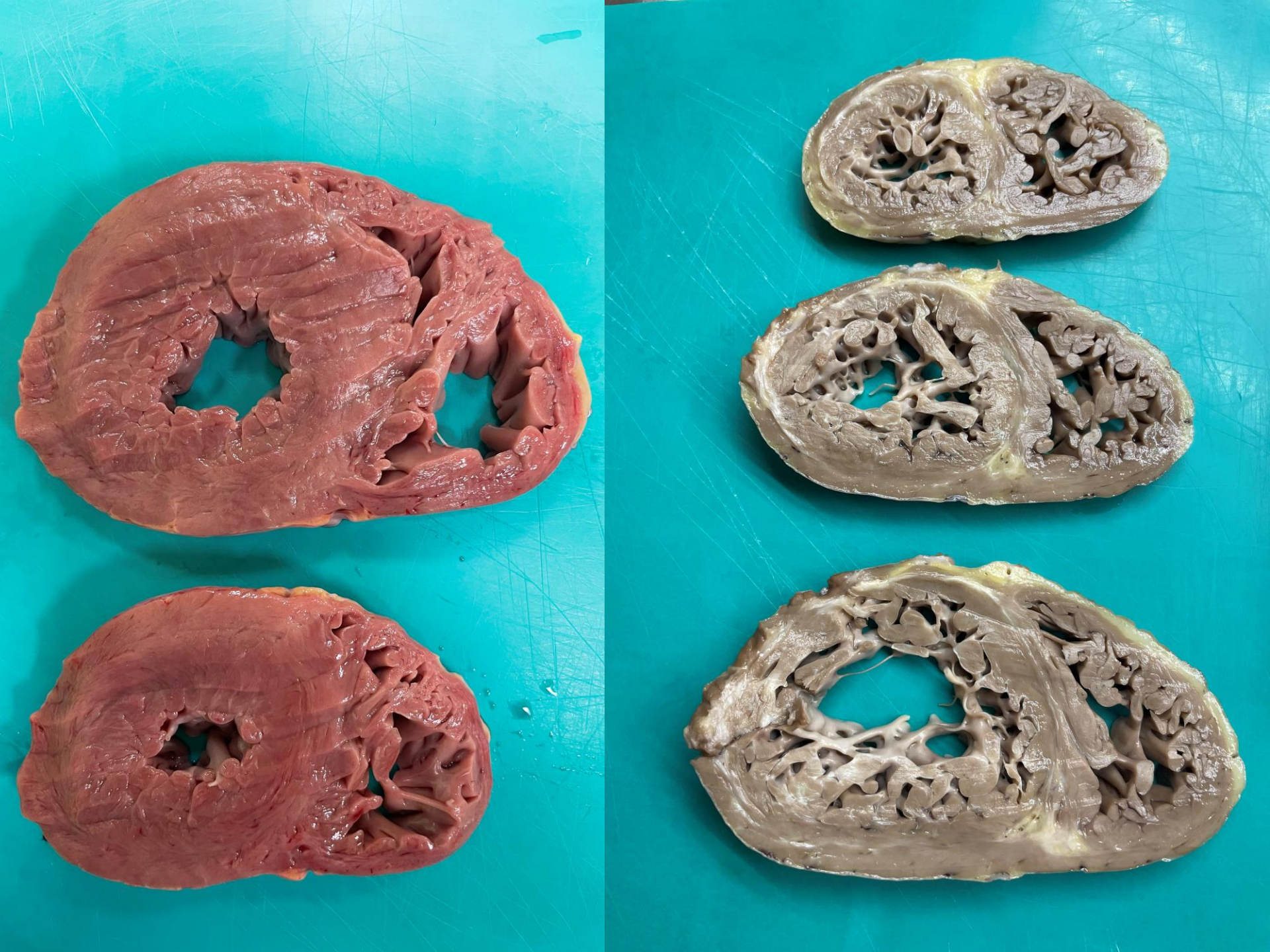
- Sampling
- Fixation
- Dehydration
- Clearing
- Embedding
- Sectioning
- Staining
- Mounting
- Observation

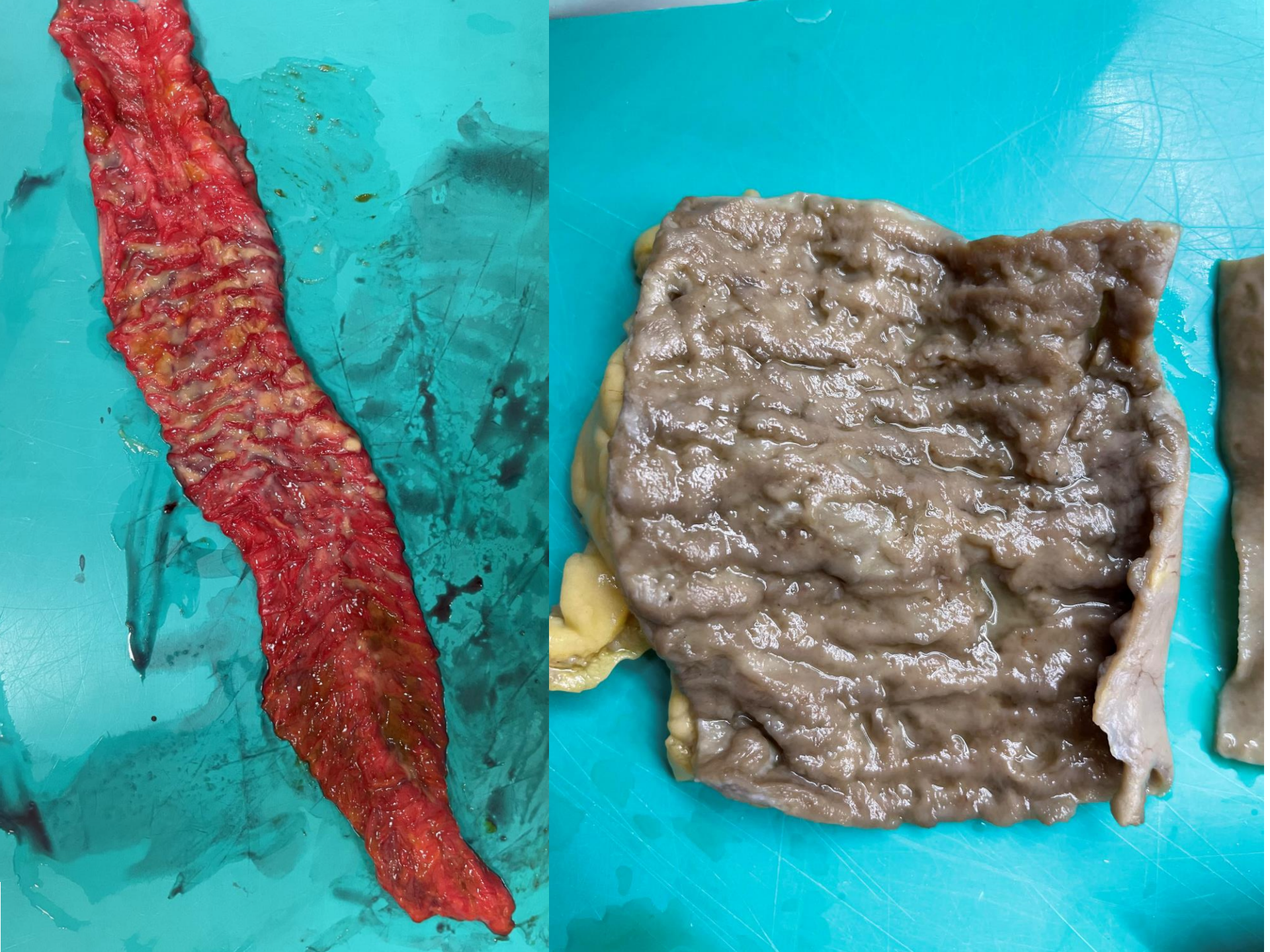
ELECTRON MICROSCOPY

- Sampling
- Fixation
- Dehydration
- Clearing
- Embedding
- Sectioning
- Contrasting
- Observation

FIXATION OF TISSUE

- artificially evoked interruption of all vital processes in the collected specimen of tissue
- coagulation of tissue proteins by chemical substances or physical factors
- formaldehyde





PARAFFIN EMBEDDING

- **DEHYDRATION**
  - increasing series of alcohol = 70 - 96% alcohol – consecutive replacement of water with alcohol
  - absolute (100%) alcohol (completing dehydration)
- **CLEARING**
  - cedar oil, xylene – replacement of the absolute alcohol with a paraffin solvent
- **PARAFFIN INFILTRATION**

  paraffin at 56 - 58°C – three baths – consecutive replacement of the clearing medium with melted paraffin
- **EMBEDDING**
  - submersion of the paraffin-infiltrated specimen with melted paraffin

tissue processor

embedding centre with cooling plate

RESIN EMBEDDING

- Dehydration
  - increasing series of alcohol = 50 - 96% alcohol – consecutive replacement of water with alcohol
  - alcohol can be diluted by 1% uranylacetate (precontrasting)
  - absolute (100%) alcohol (completing dehydration)
- Clearing (if necessary, e.g. in epoxy resins)
  - propylene oxide - replacement of the absolute alcohol with a resin solvent
- Infiltration with embedding medium
  - replacement of the clearing agent with liquid resin
- Embedding with resin in gelatine capsules
- Polymerization - hardening
  - heat (60°C) – most resins
  - UV light at room temperature or in freezer – some acrylic resins

resin-embedding tissue processor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SECTIONING

- **MICROTOME**
  - sliding or rotary
  - steel knives or blades
  - for paraffin (celoidine, celodal, gelatine) blocks
- **ULTRAMICROTOME**
  - glass or diamond knives
  - for resin blocks
- **CRYOSTAT AND FREEZING MICROTOME**
  - steel knives or blades
  - for frozen specimens

sliding microtome

rotary microtome

Ultramicrotome

CRYOSTAT – combination of rotary microtome a freezing box

manual

motorized

USING OF FREEZING TECHNIQUES

- **rapid intraoperative biopsies – mainly evaluation of tumors**
- **lipid staining**
- **lipid histochemistry**
- **enzyme histochemistry**
- **immunohistochemistry – vulnerable antigens**
- **some fluorescence methods**
- **some impregnation methods**

STAINING

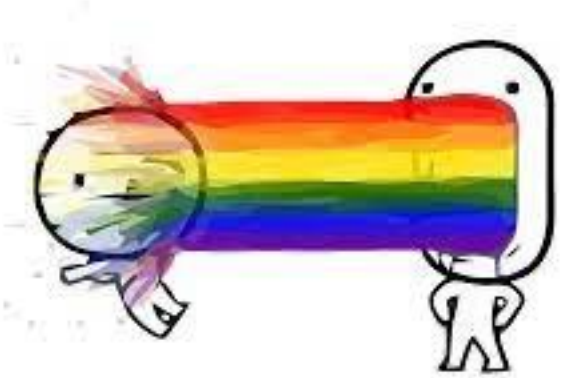
Interaction between a dye (staining agent) and a certain tissue component.

- **1/ synoptic staining – all components of the specimen are stained, based on the acid-base affinity**
  - Basic dyes – haematoxylin (various types), thionine, azocarmine, toluidine blue, nuclear red, methylene green,……
  - Acidic dyes – eosin, erythrosin, light green, acid fuchsin, orange G, aniline blue, picric acid,……

2/ special staining - highlighting of assessed structures by a dye with specific affinity

Basic staining procedure
(of paraffin section)

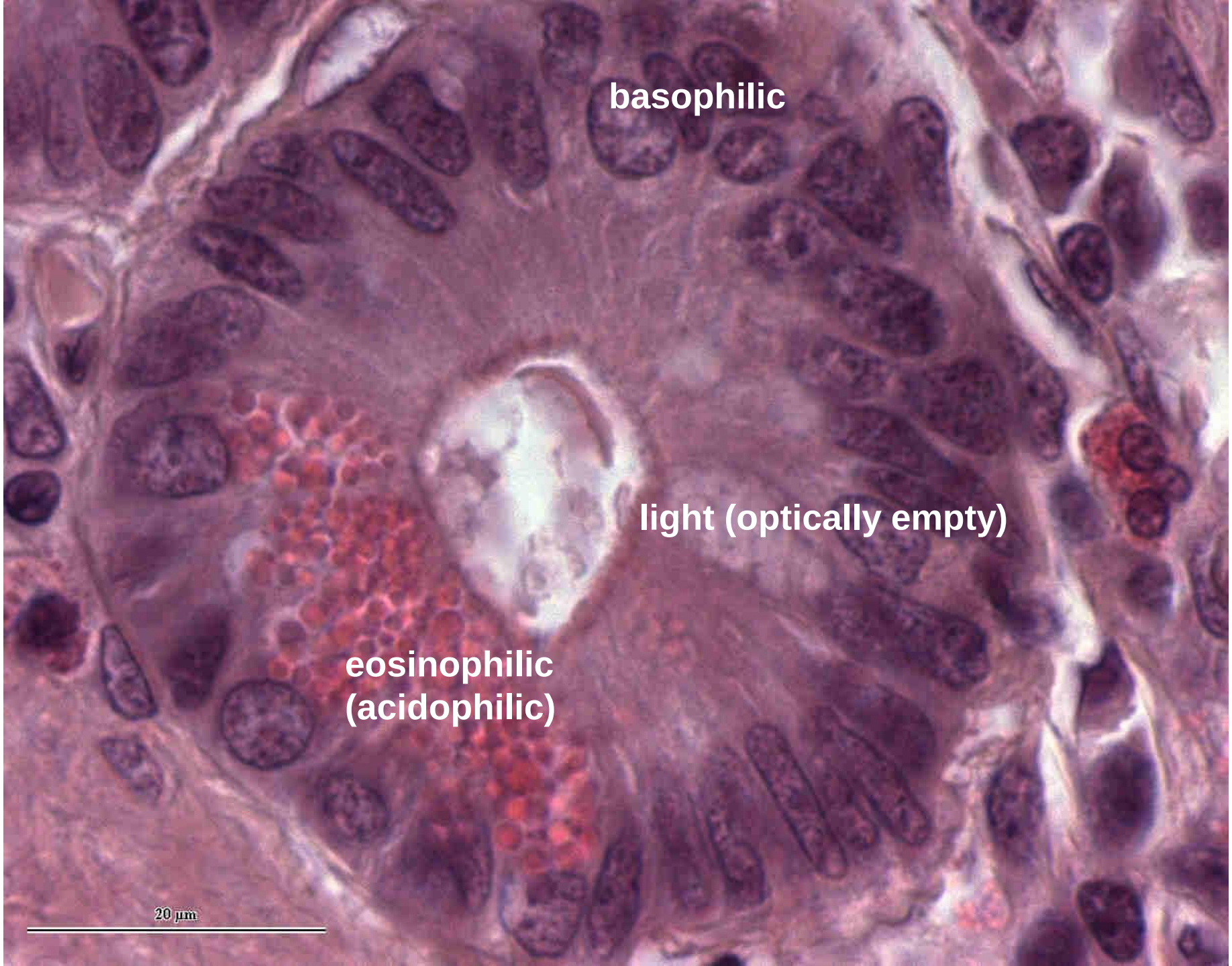
- **slide deparaffination**
- **slide rehydration**
- **staining**
- **slide dehydration**
- **slide clearing before mounting**




EOSIN
ALKOHOL
KARBOXYLEN
XYLEN
XYLEN

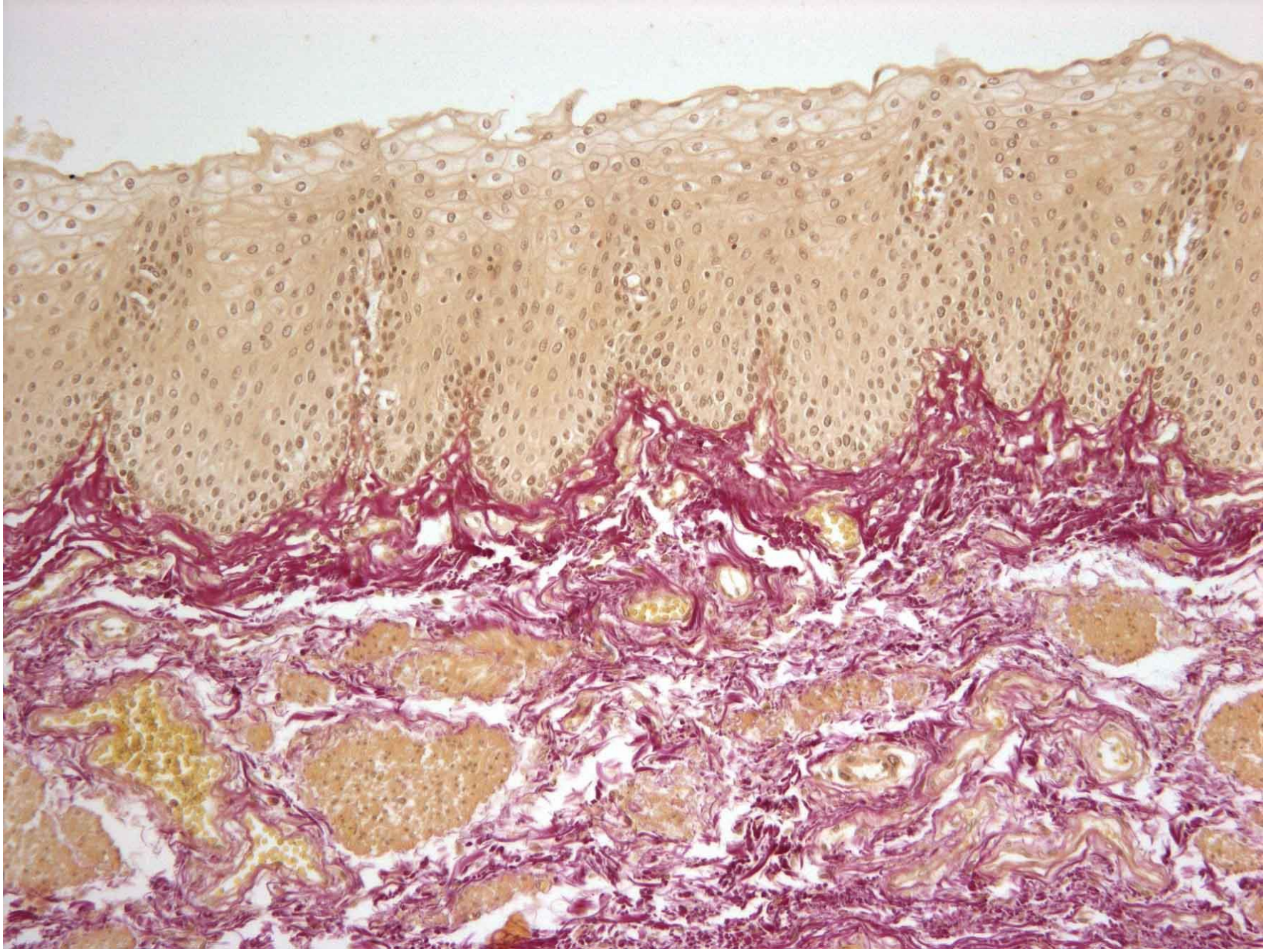
MAKING A PERMANENT HISTOLOGICAL SLIDE STAINED WITH HAEMATOXYLIN AND EOSIN (H&E)

- **DEPARAFFINATION AND REHYDRATION**
  - xylene (2 baths) - dissolving of paraffin
  - 100% alcohol - washing off the dissolved paraffin and dissolvent
  - 96% - 70% alcohol - slow rehydration
  - water - washing off the alcohol
- **STAINING**
  - **haematoxylin (basic dye)** - staining of **acid (basophilic)** components of tissue **(nuclei, ribosomes)**
  - washing
  - **eosin (acid dye)** - staining of **basic (acidophilic, eosinophilic)** components of tissue **(proteins)**
  - washing (water)
- **DEHYDRATION**
  - 70% - 100% alcohol - gradual replacement of water
- **CLEARING**
  - xylene (2 baths) - replacement of alcohol with dissolvent of mounting medium
- **MOUNTING**
  - Solacryl BMX - covering of the slide with the coverslip

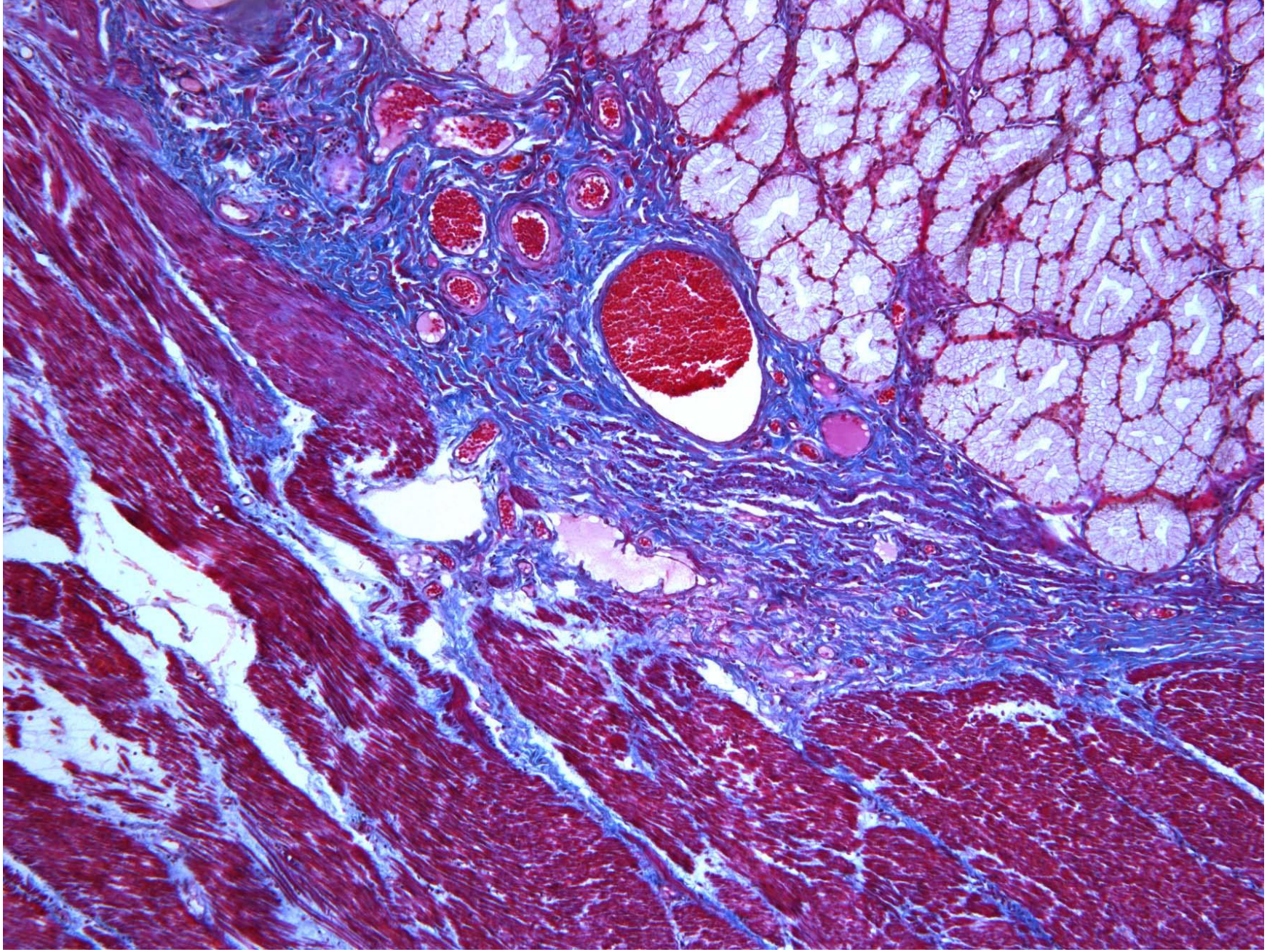

basophilic
light (optically empty)
eosinophilic
(acidophilic)
20 µm

OTHER EXAMPLES OF SYNOPTIC STAININGS

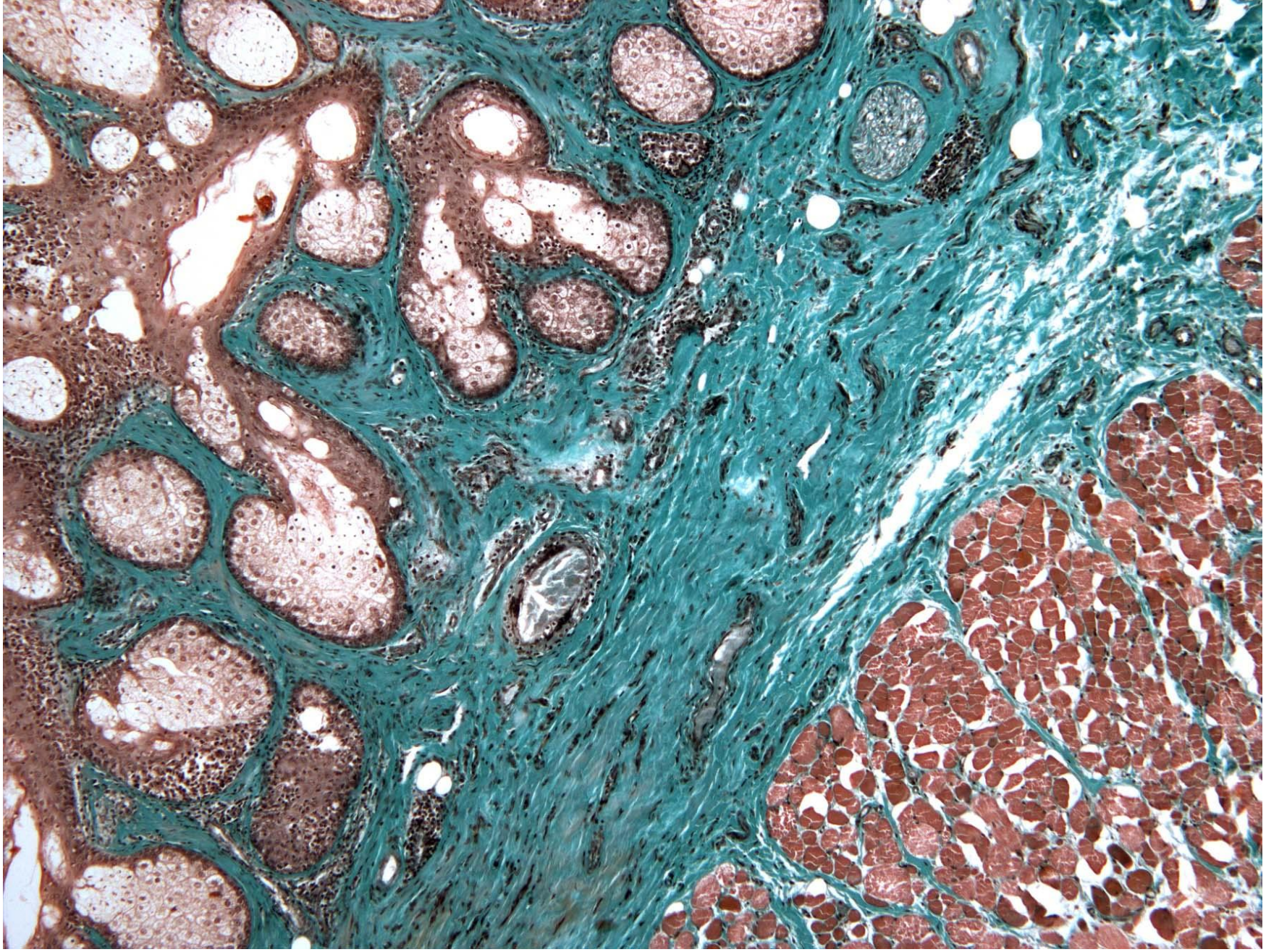
Weigert - van Gieson	iron haematoxylin + acid fuchsin + picric acid	nuclei brown, collagen red, muscle yellow
Masson's trichromes -yellow	haematoxylin + erythrosin + saffron	nuclei blue, collagen yellow, muscle red
-blue	iron haematoxylin + acid fuchsin + aniline blue	nuclei brown to black, collagen blue, muscle red
-green	iron haematoxylin + acid fuchsin + orange G + light green	nuclei brown to black, collagen green, muscle red
AZAN	azocarmine + aniline blue + orange G	nuclei red, collagen blue, muscle red



Weigert - van Gieson



blue trichrome

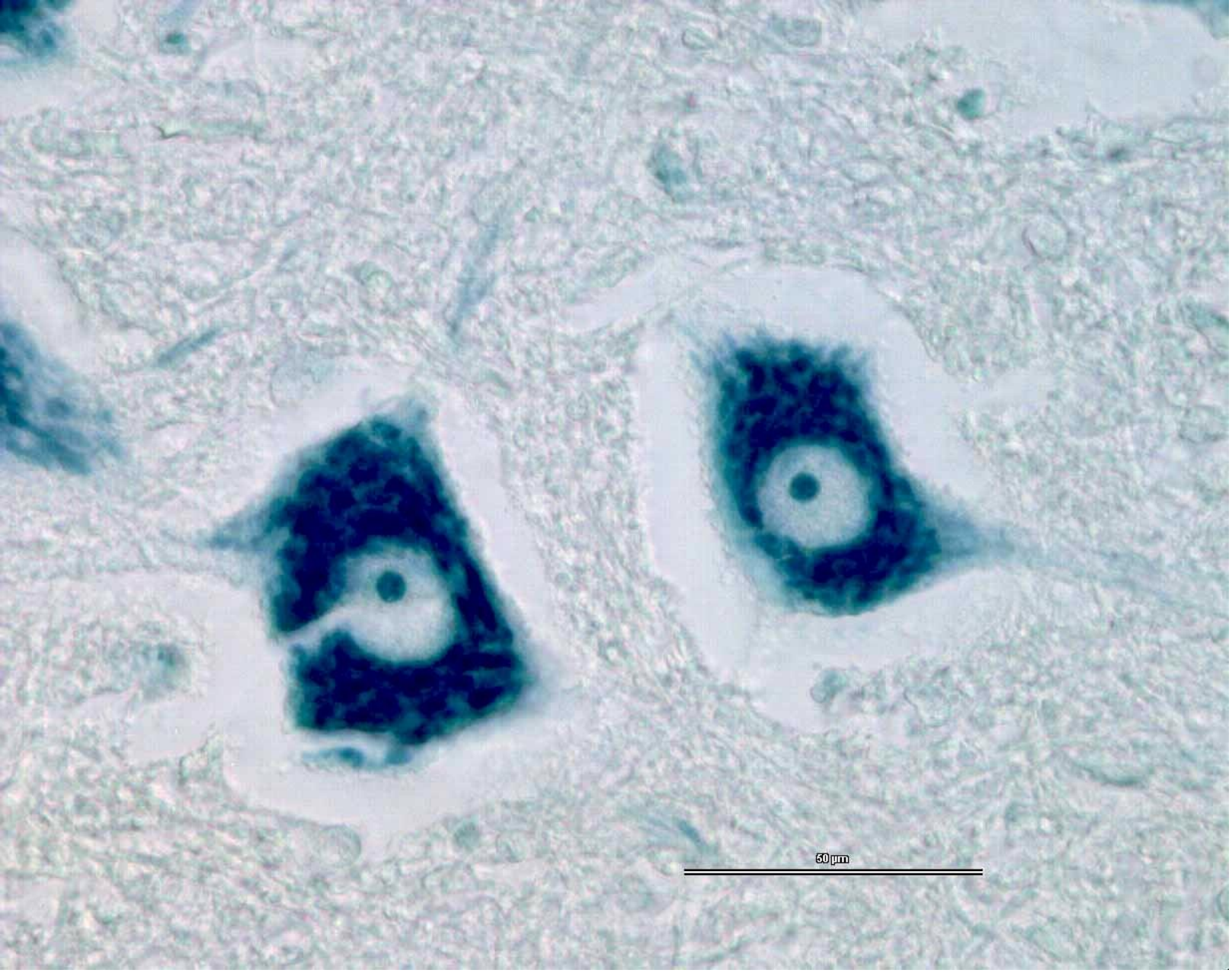


green trichrome

SPECIAL STAINING

-highlighting of searched structures with a **dye** of specific affinity
-no colour chemical reaction takes place (dye does not change its original colour)

-cytological stainings – staining of selected intacellular structures
-selective stainings – staining of selected structures or substances regardless of their location
-impregnation methods – reduction of metals (Ag, Au, Os) on selected structures



toluidine blue **Nissl substance**

EXAMPLES OF SELECTIVE STAININGS

elastics - orcein, aldehyde fuchsin, resorcine fuchsin

mucus, glycogen, glycoaminoglycans (GAG) - alcian blue, mucicarmine, Best carmine

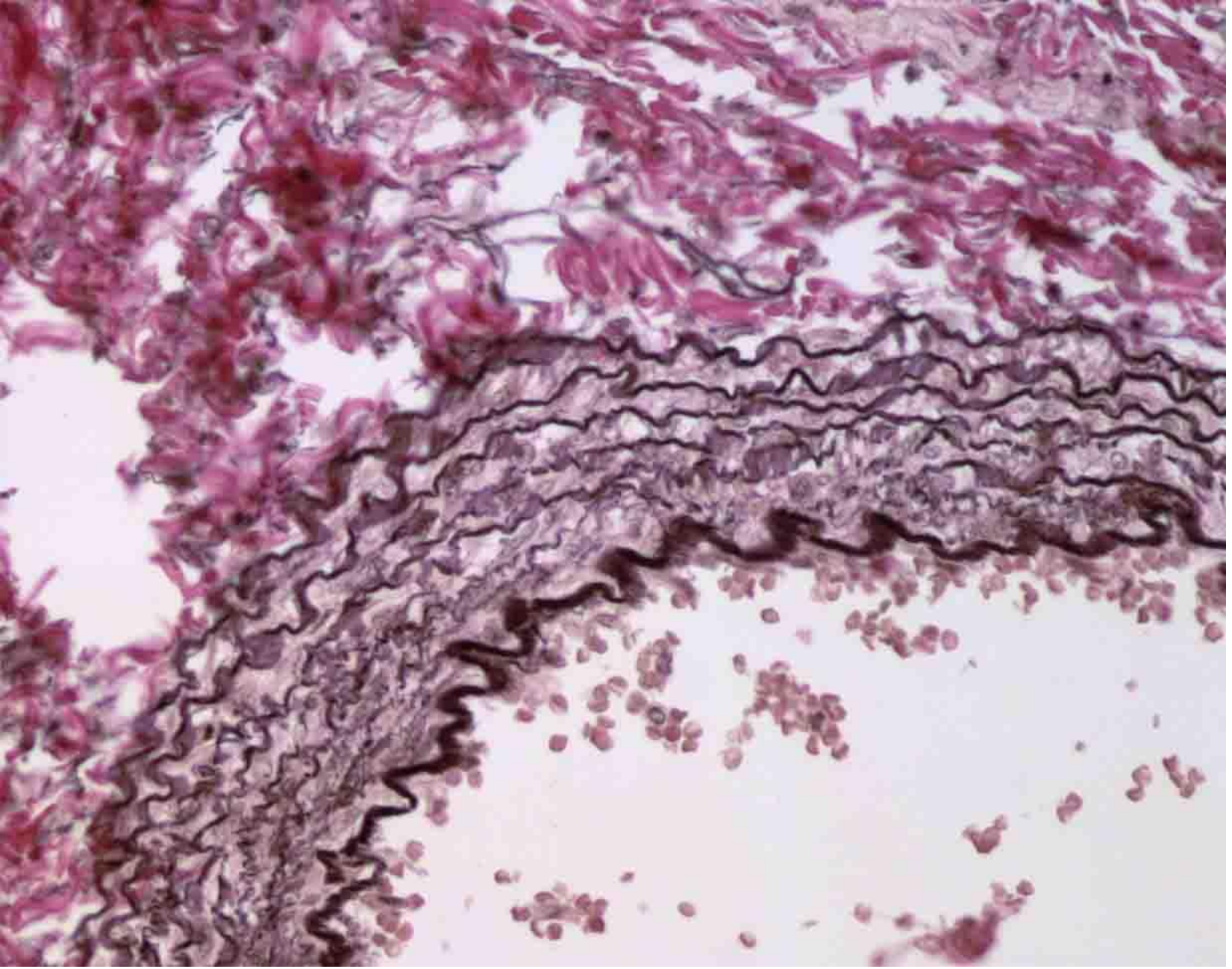
neutral lipids – Oil red O, Sudan black B, Sudan III, scarlet – avoidance of lipid dissolvents (frozen sections)

myelin (phospholipids) – luxol blue, Spielmeyer haematoxylin

amyloid - Congo red, methylene violet, Saturn red

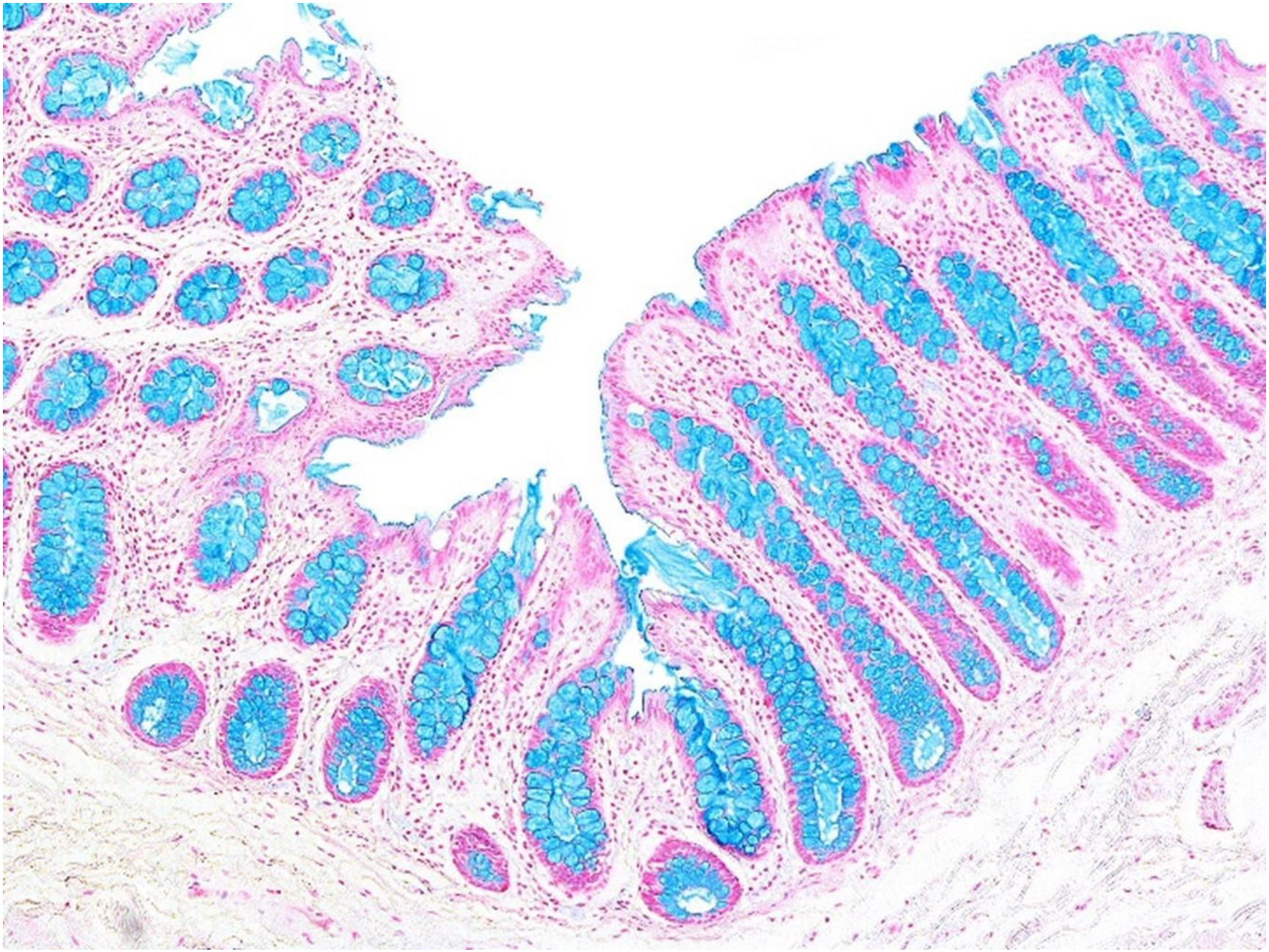
fibrin – Weigert staining

Etc. …

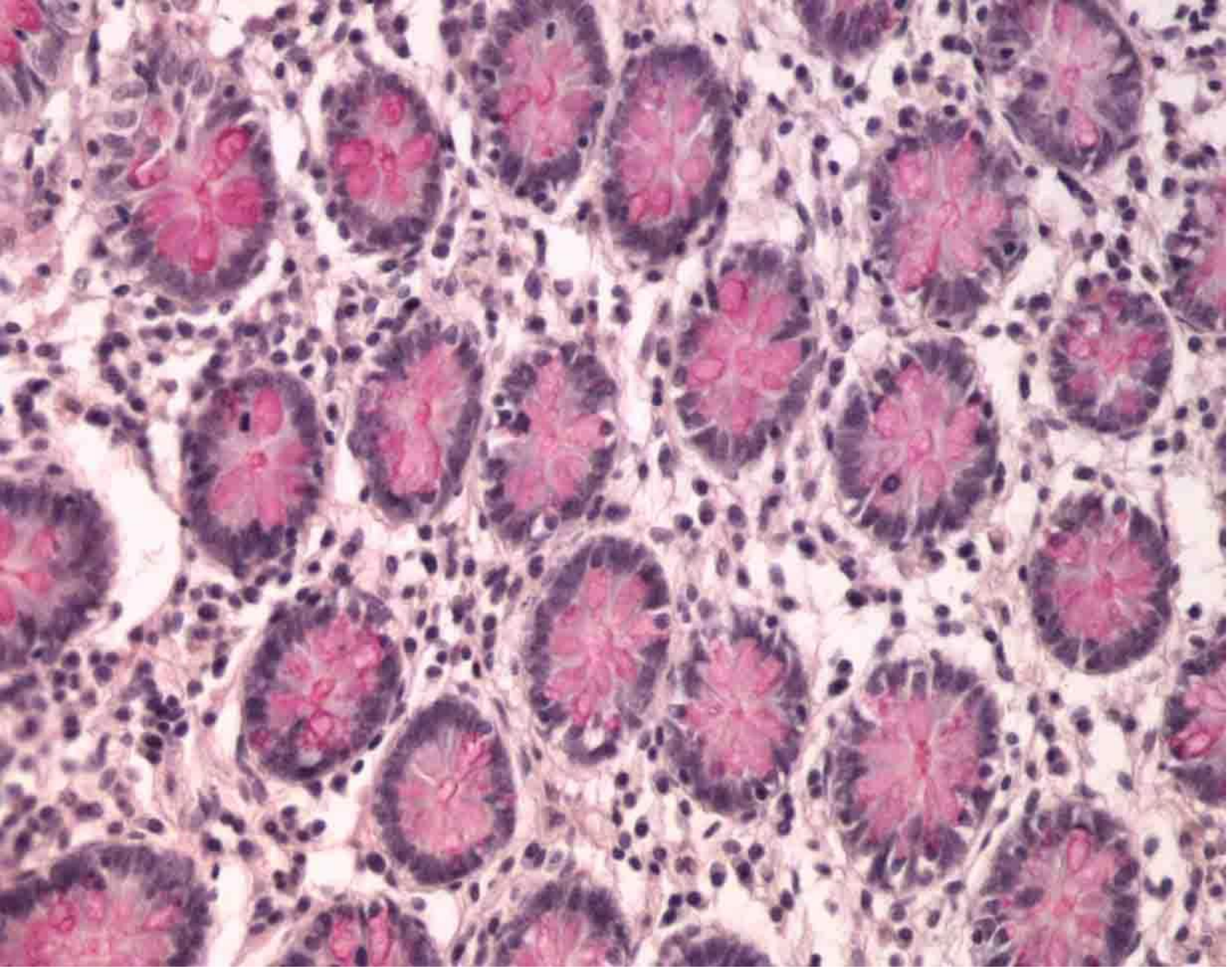


resorcine fuchsin

elastics

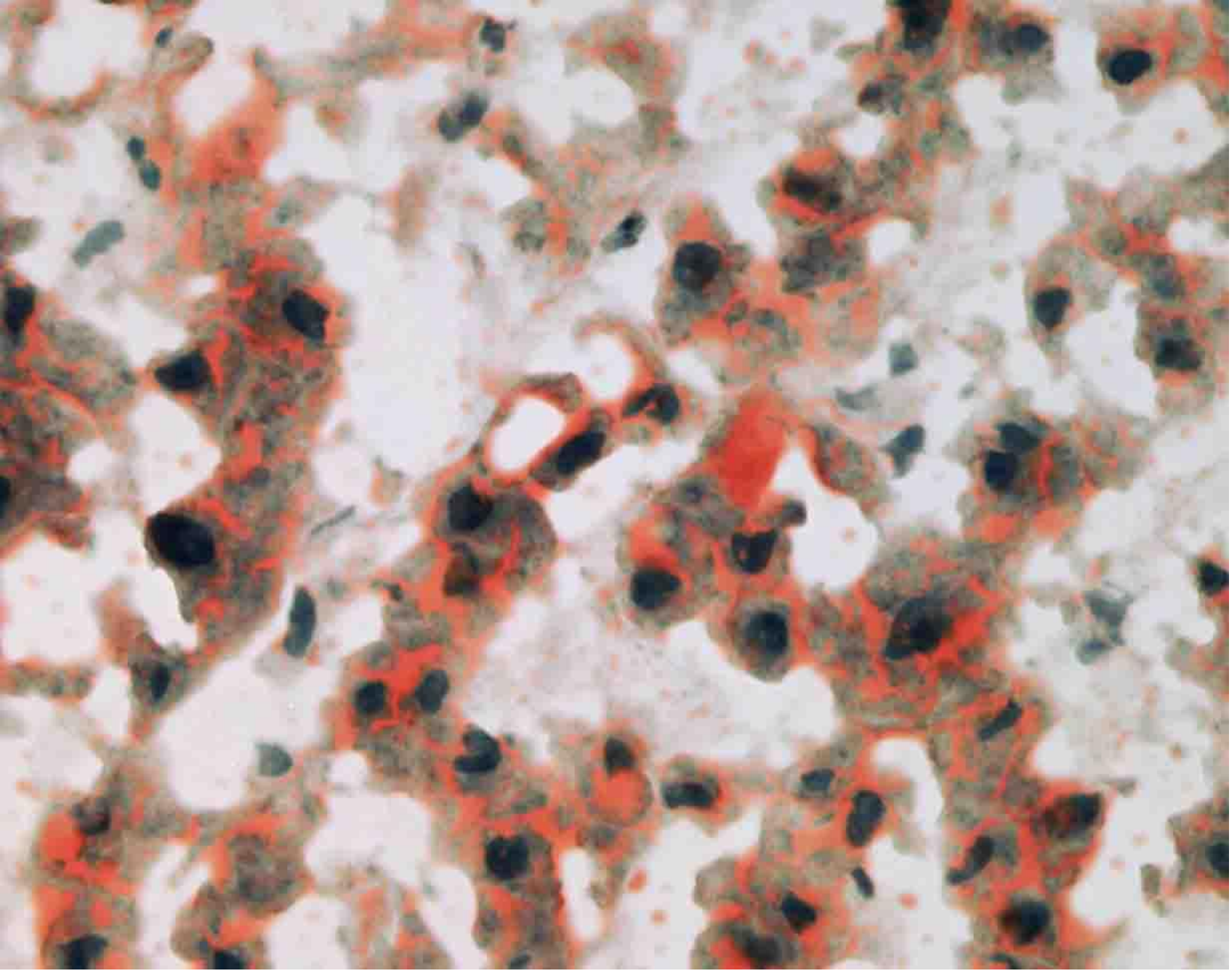


alcian blue pH 2.5 **acidic mucopolysaccharides**



mucicarmine

mucins

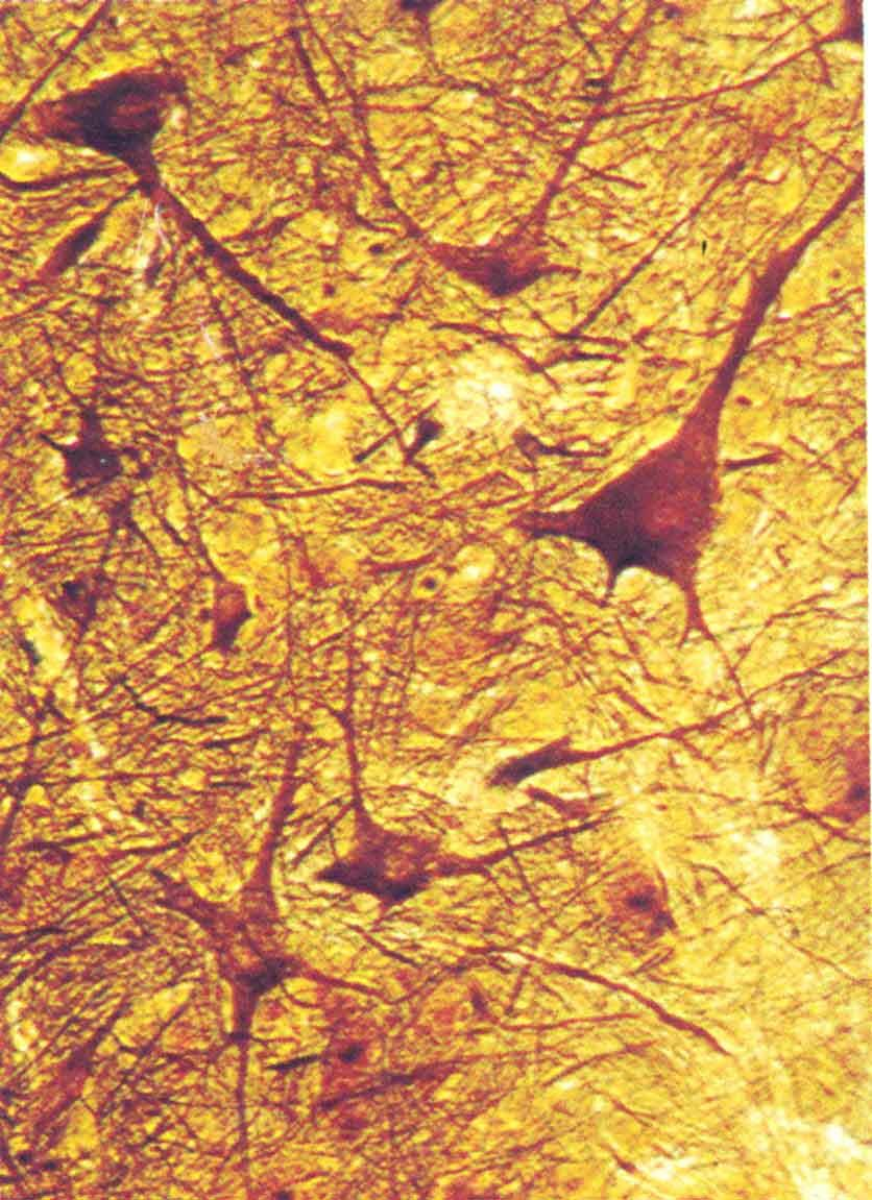


Oil red O

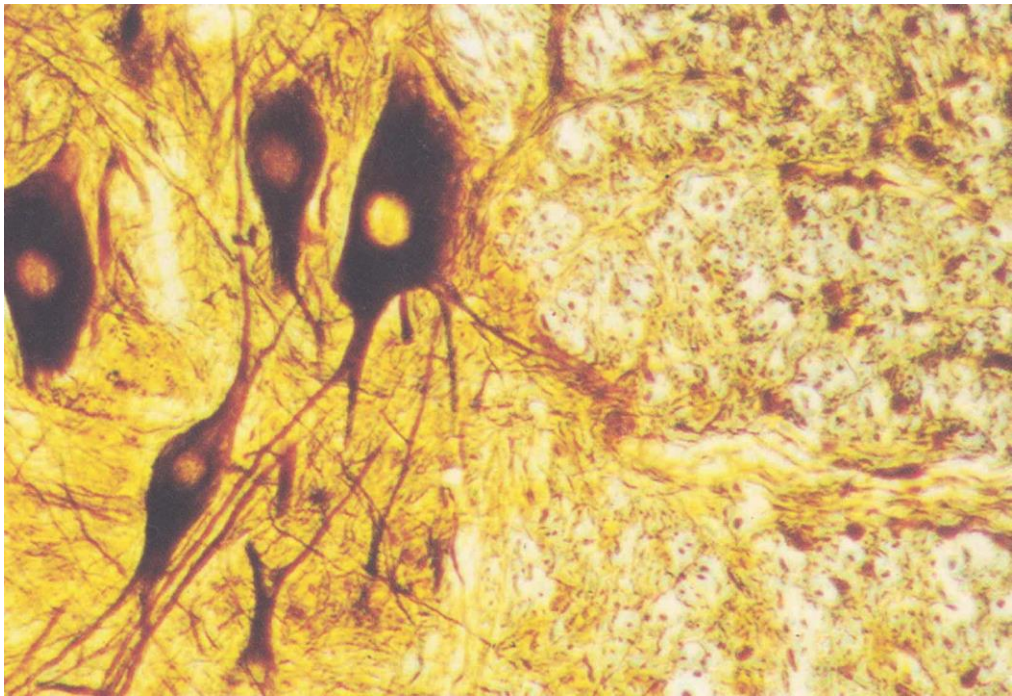
neutral lipids

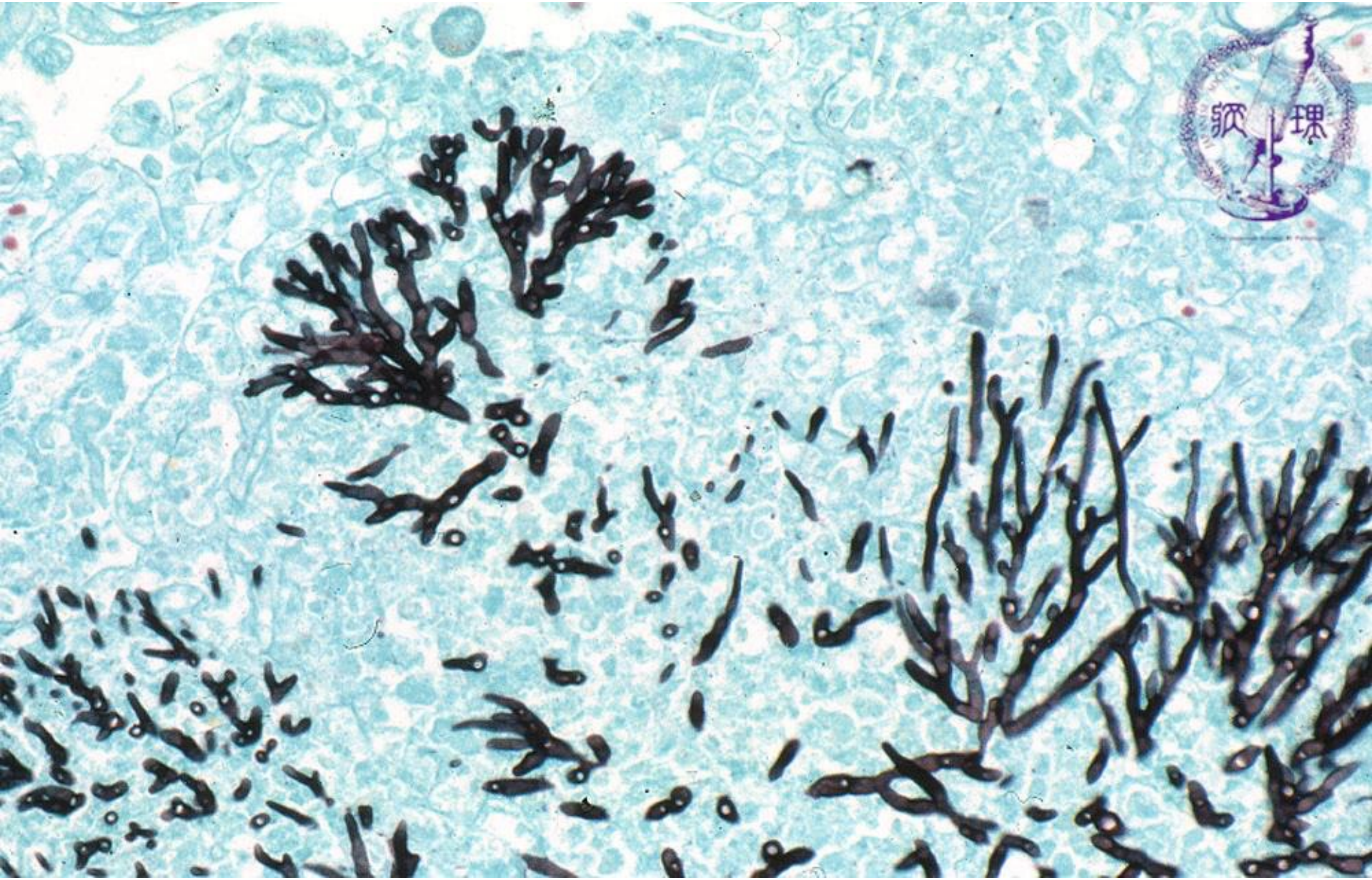
EXAMPLES OF IMPREGNATION

Gömöri, Foot	reticular fibres
Hortega, Cajal	astrocytes
Penfield	oligodendrocytes
Bielschowski	nerve fibres
Grimelius	cells of DNES
Grocott	hyphes of fungi



neuron impregnation

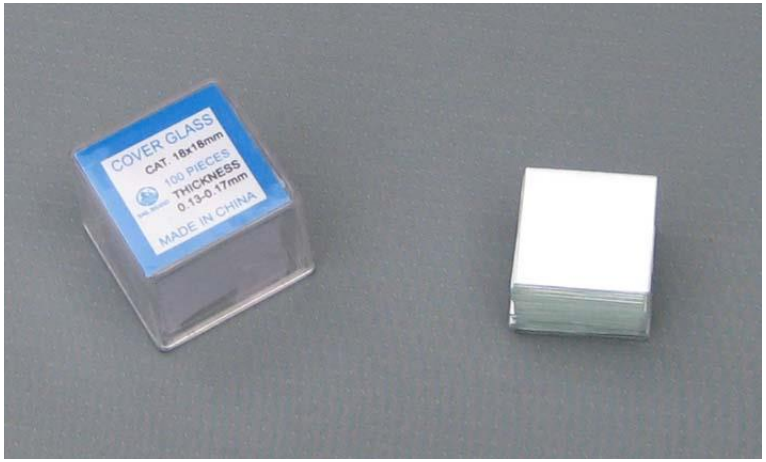




Mycosis (Aspergilus)- Grocott

MOUNTING

- cover glass

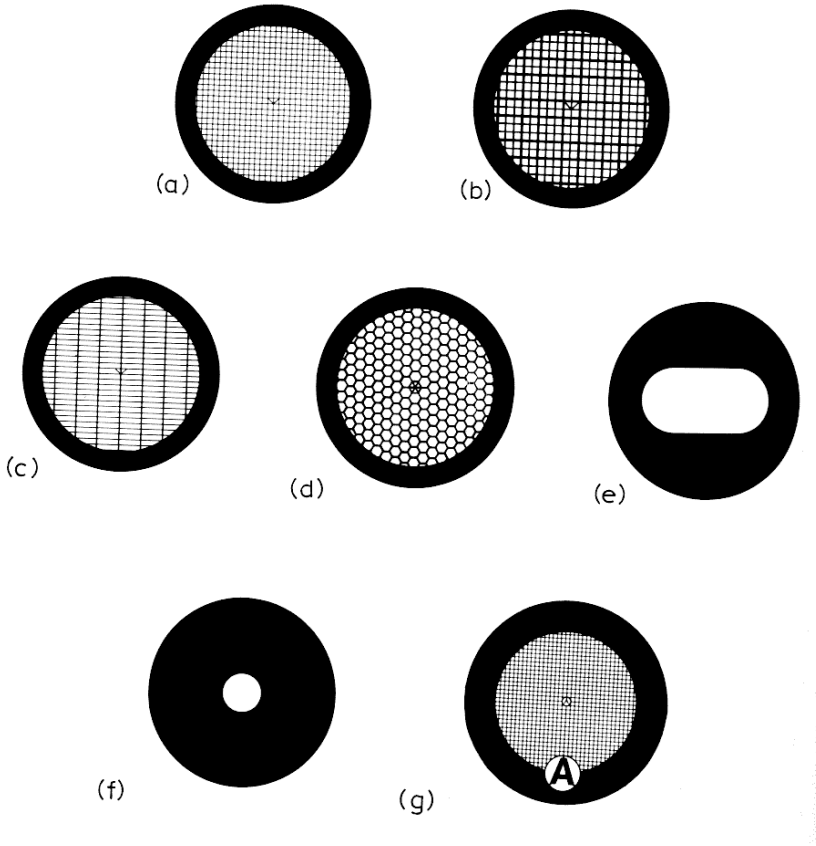


- xylene-soluble media
  - acrylic resins (solacryl BMX)
  - Canada balsam

- water-soluble media
  - glycerol-gelatine
  - glycerol


1. Epithelial tissue
HHS-01 Simple columnar epithelium, in sec. of human intestine
HHS-02 Simple cuboidal epithelium, in t.s. of human part bladder
HHS-03 Columnar epithelium, in t.s. of human thyroid gland
HHS-04 Simple ciliated columnar epithelium, in t.s. of oviduct
HHS-05 Pseudostratified ciliated columnar epithelium, trachea, t.s.
HHS-06 Stratified, non-cornified squamous epithelium, oesophagus sec.
HHS-07 Transitional epithelium, isolated cells from human mouth, smear
HHS-08 Squamous epithelium, silver stained
HHS-09 Golgi apparatus, male
HHS-10 Normal human chromosome smear, male
HHS-11 Normal human chromosome smear, female
2. Connective tissue
HHS-12 Loose connective tissue of Human sec.
HHS-13 Dense connective tissue of Human sec.
HHS-14 Adipose tissue of Human sec.
HHS-15 Hyaline cartilage of Human sec.
HHS-16 Fibrous cartilage of Human sec.
HHS-17 Elastic cartilage of Human sec.
HHS-18 Human bone grinding w.m.
HHS-19 Joint of human foetus, t.s.
HHS-20 Finger tip from human foetus, sagittal t.s. of nail development
3. Muscular tissue
HHS-21 Skeletal muscle of Human t.s.and l.s. (hematoxylin stain)
HHS-22 Skeletal muscle of Human sec. (hematoxylin stain)
HHS-23 Cardiac muscle of Human t.s.and l.s. (H.E. stain)
HHS-24 Cardiac muscle of Human sec. (H.E. stain)
HHS-25 Smooth (involuntary) muscle, human t.s. and l.s.
4. Nervous system
HHS-26 Spinal cord of Human t.s.
HHS-27 Spinal cord smear show general structure
HHS-28 Spinal ganglion, human t.s. H.E. stained
HHS-29 Sympathetic ganglion, human t.s. H.E. stained
HHS-30 Cerebrum of Human sec. (HE)
HHS-31 Cerebellum of Human sec. (HE)
5. Circulation system
HHS-32 Blood smear, human, Giemsa stain
HHS-33 Blood smear, human, H.E.
HHS-34 Human Medium-sized artery and vein sec.
HHS-35 Artery, human, t.s. H.E. stained
HHS-36 Vein, human, t.s. H.E. stained
6. Lymphatic system
HHS-37 Lymph node, human t.s.
HHS-38 Spleen, human t.s.
HHS-39 Thymus of Human sec.
HHS-40 Tonsil (Tonsilla palatina), human t.s.
HHS-41 Red bone marrow smear, Giemsa stained
7. Endocrine system
HHS-42 Thyroid gland of Human sec.
HHS-43 Parathyroid gland of Human sec.
HHS-44 Adrenal gland of Human sec.
HHS-45 Pituitary gland (Hypophysis), human t.s.
HHS-46 Pancreas with islets of Langerhans, human, sec.
8. Digestive system
HHS-47 Lip, human t.s.
HHS-48 Soft palate, human t.s.
HHS-49 Hard palate, human t.s.
HHS-50 Tongue, human, sec. with filiform papillae
HHS-51 Tongue, human, t.s. with fungiform papillae
HHS-52 Oesophagus, human t.s.
HHS-53 Lives of cirr. silver stained show bile canals.silve
HHS-54 Stomach, cardiac region, human t.s.
HHS-55 Stomach, fundic region, human t.s.
HHS-56 Stomach, pyloric region, human t.s.
HHS-57 Duodenum of Human sec.
HHS-58 Jejunum of Human sec.
HHS-59 Ileum of Human sec.
HHS-60 Vermiform appendix, human t.s.
HHS-61 Colon of Human sec.
HHS-62 Rectum, human t.s.
HHS-63 Parotid gland of Human sec.
HHS-64 Submaxillary gland, human t.s.
HHS-65 Sublingual salivary gland, human t.s.
HHS-66 Liver, human t.s.
HHS-67 Gall bladder, human t.s.
9. Respiratory system
HHS-68 Trachea of Human t.s.
HHS-69 Trachea of Human t.s.
HHS-70 Epiglottis, human sec.
HHS-71 Human Lung sec.
10. Urinary system
HHS-72 Urinary bladder of Human sec.
HHS-73 Ureter of Human t.s.
HHS-74 Kidney, human t.s.
11. Female Reproductive system
HHS-75 Ovary, human, t.s.
HHS-76 Oviduct (Ballopian tube), t.s.
HHS-77 Human Uterus (proliferative phase) sec.
HHS-78 Human Uterus (secretory phase) sec.
HHS-79 Uterine cervix of Human sec.
HHS-80 Placenta of Human sec.
HHS-81 Umbilical cord (navel string), human t.s.
HHS-82 Vagina of Human sec.
12. Male Reproductive system
HHS-83 Penis of Human sec. (infant)
HHS-84 Testis of Human sec.
HHS-85 Sperm smear, human
HHS-86 Epididymis, human t.s.
HHS-87 Prostate of Human sec.
HHS-88 Spermatic cord (Ductus deferens) of human t.s.
HHS-89 Seminal vesicle (glandula vesiculosa), human t.s.
13. Sensory organs
HHS-90 Eyeball, Human sec.
HHS-91 Wallate papillae with taste buds, human t.s.
HHS-92 Olfactory epithelium, human t.s.
HHS-93 Tactile corpuscle of Human sec.
HHS-94 Lamellar corpuscle of Human sec.
14. Integument (Skin)
HHS-95 Scalp, vertical t.s. shows hair follicles human, H.E. stained
HHS-96 Skin from armpit with apocrine glands, vertical t.s.
HHS-97 Eyelid, human, t.s.
HHS-98 Mammary gland, active, human t.s.
HHS-99 Mammary gland, resting, human t.s.
HHS-100 Hair of Human w.m.
HAPPY
HHS-74 Kidney, human t.s.
HAPPY SCIENCE www.ihappysci.com
HHS-84 Testis of Human sec.
HHS-33 Blood smear, human, H.E.
HAPPY SCIENCE www.ihappysci.com
HHS-67 Duodenum of Human sec.

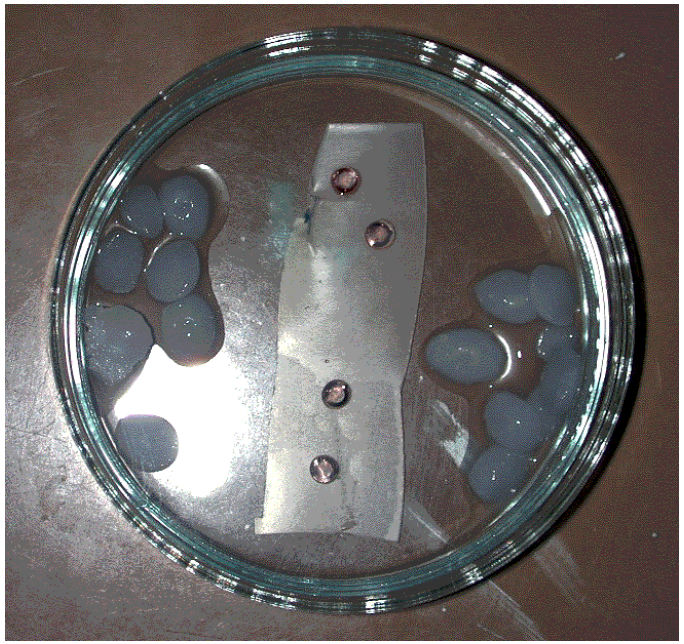
TEM - GRIDS

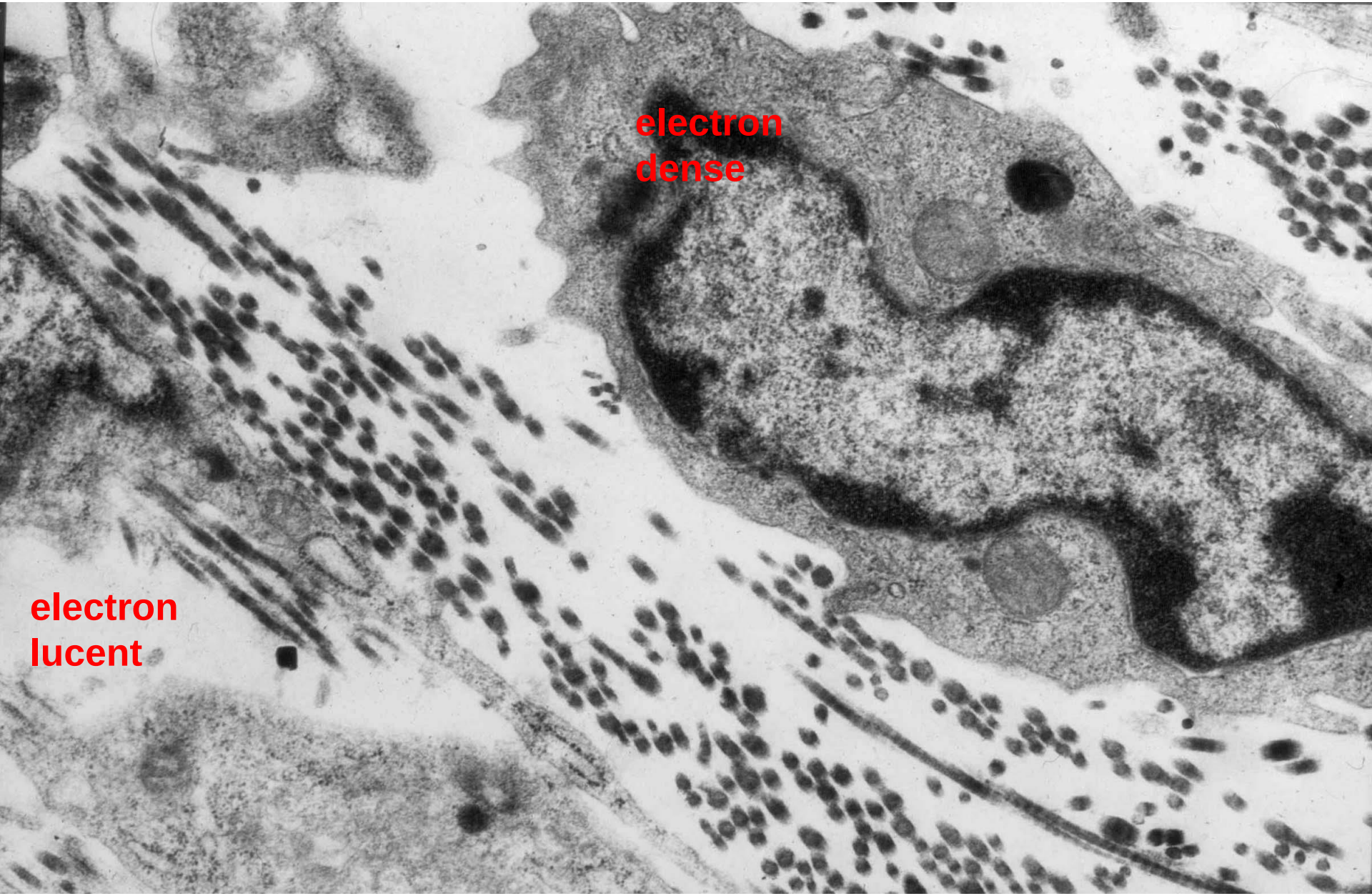


- grids can be naked or
- coated with a support film (Formvar – polyvinylformaldehyde) and evaporated carbon layer

CONTRASTING
(for TEM)

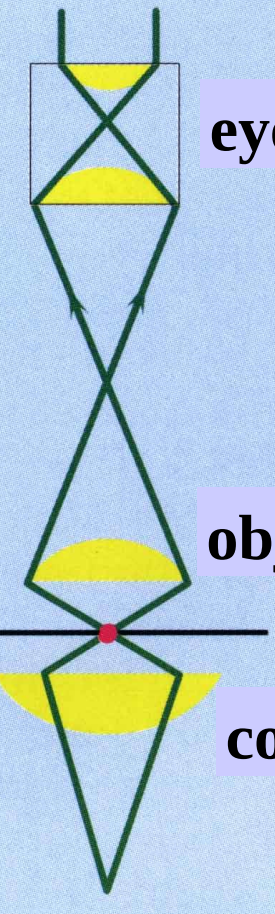
- binding of atoms of heavy metals to structures of an observed object
- grids are laid on drops of metal salts solutions
- mostly used salts:
  - uranyl acetate
  - lead citrate




electron
dense
electron
lucent

OBSERVATION

- Direct observation
- Photography
- Morphometry
- Stereology
- 3D reconstructions
- Image analysis

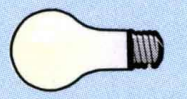


eyepiece

objective

specimen

condenser

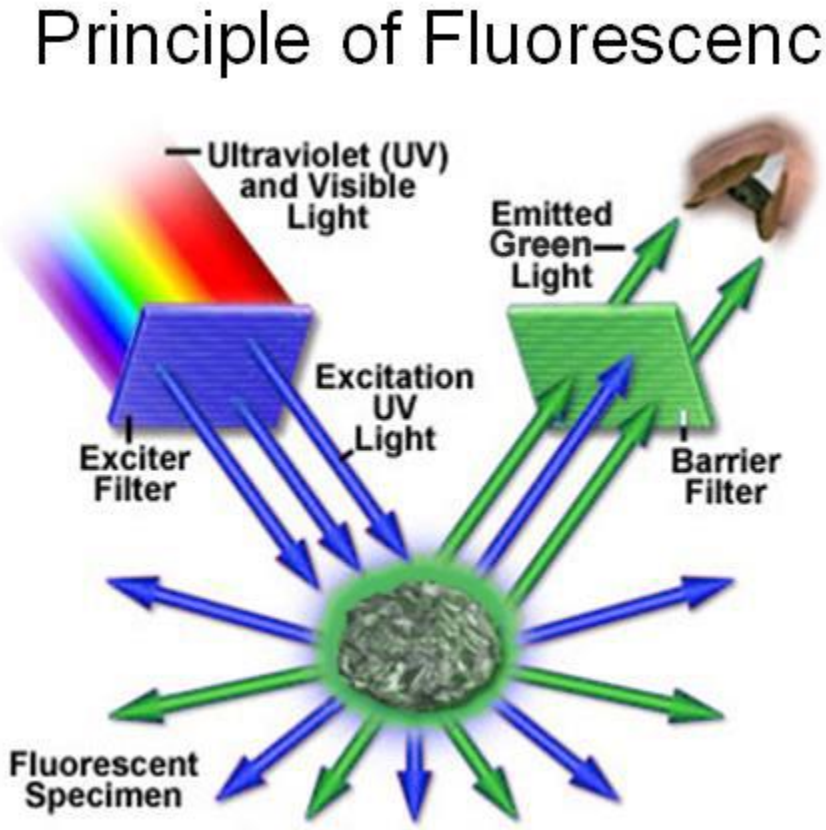


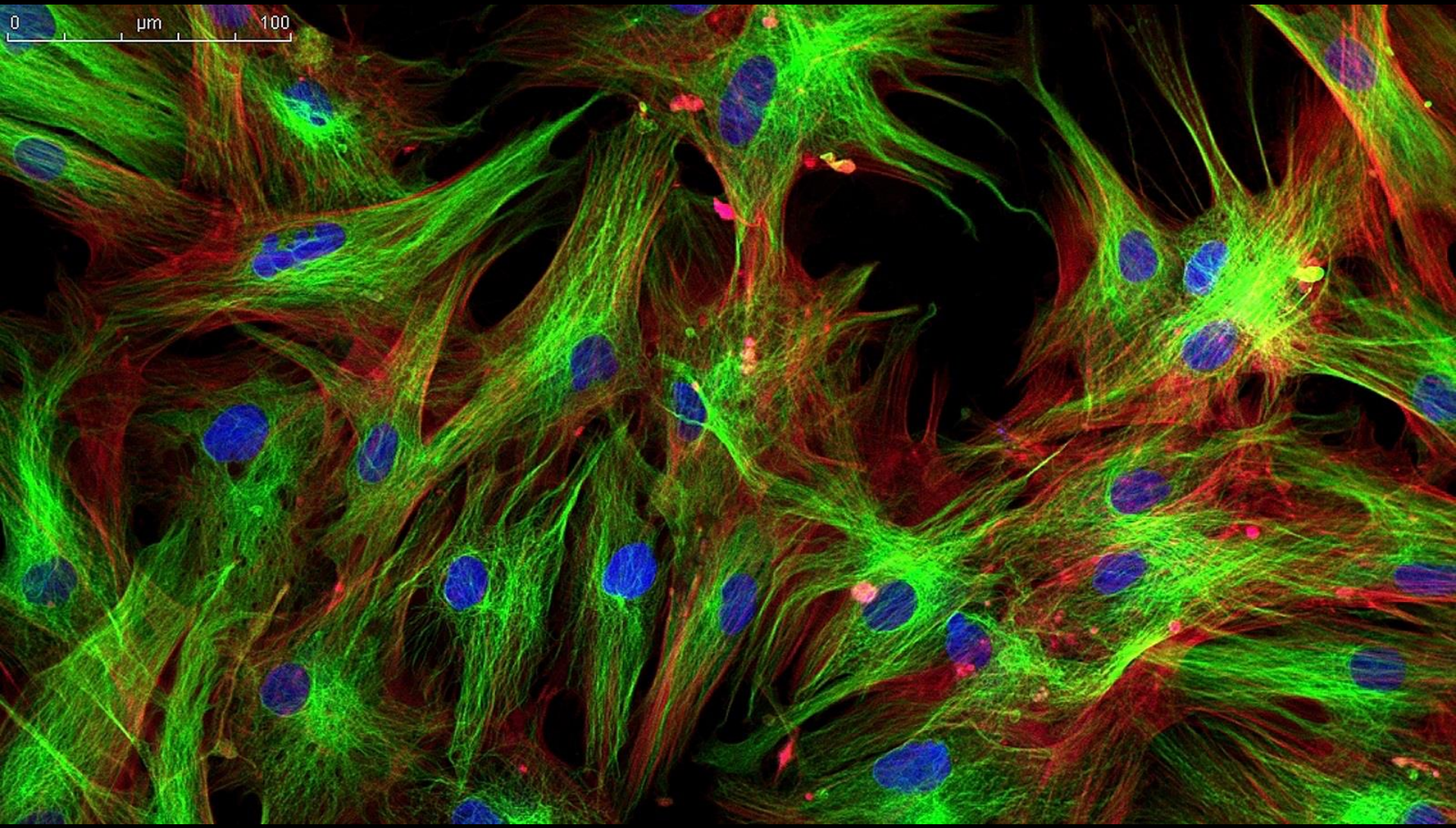
light source

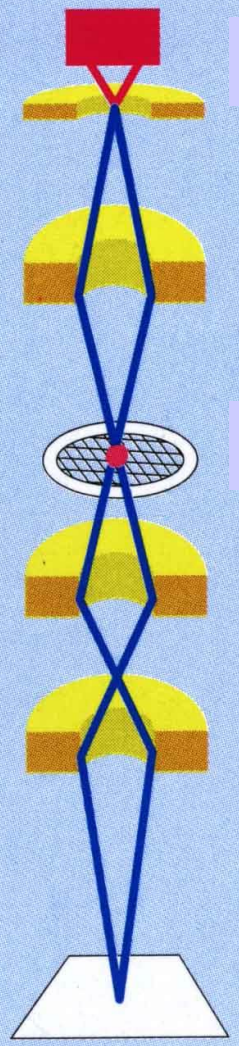
Transmission (bright-field) light microscope

Fluorescence microscopy

- **Fluorescence –** ability of some substances(fluorochromes) to respond to the absorption of excitation light by emission of light having a longer wavelength




0
µm
100



electron gun (cathode)

condenser lens

specimen

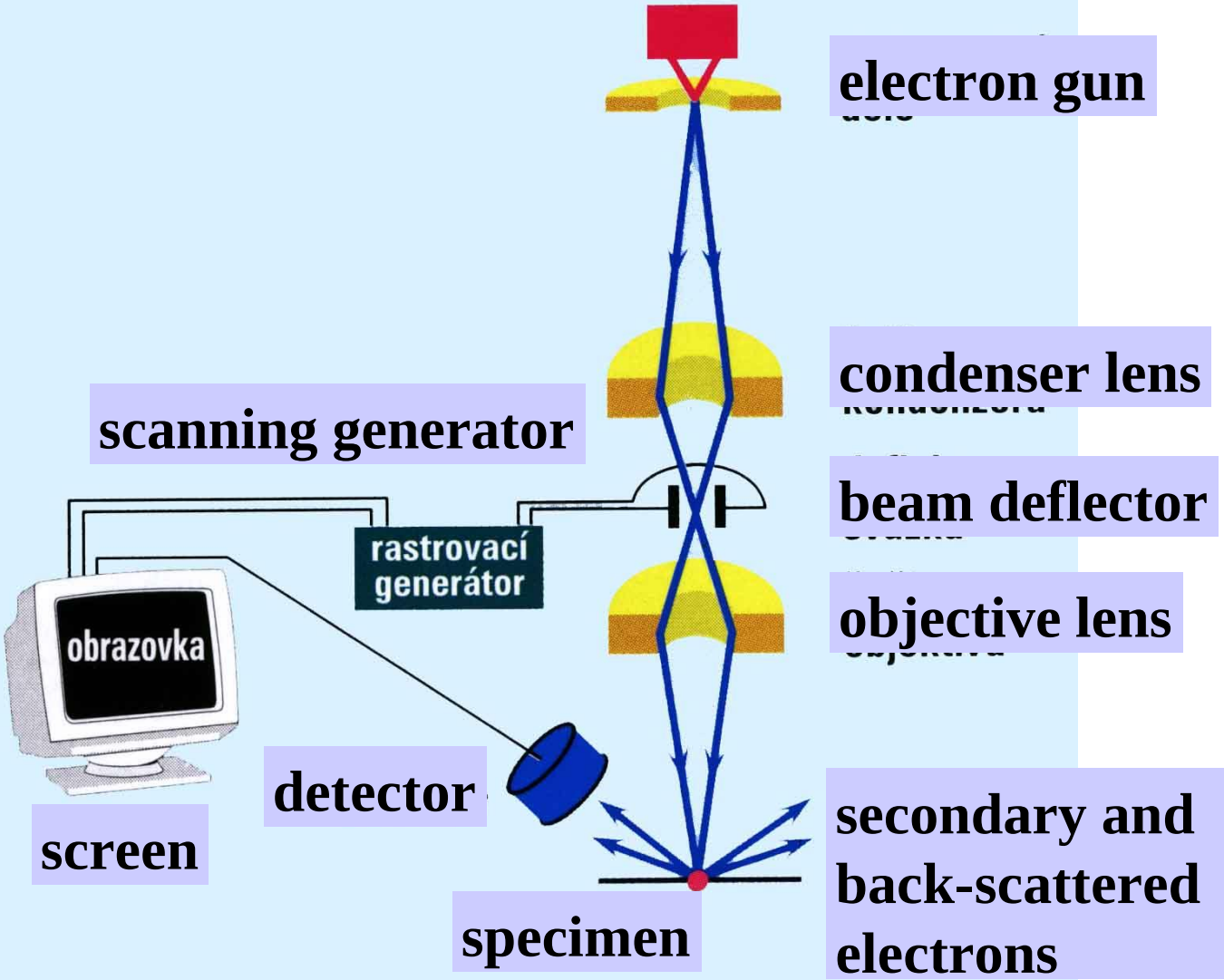
objective lens

projector lens

fluorescent screen or photographic emulsion

Transmission electron microscope (TEM)

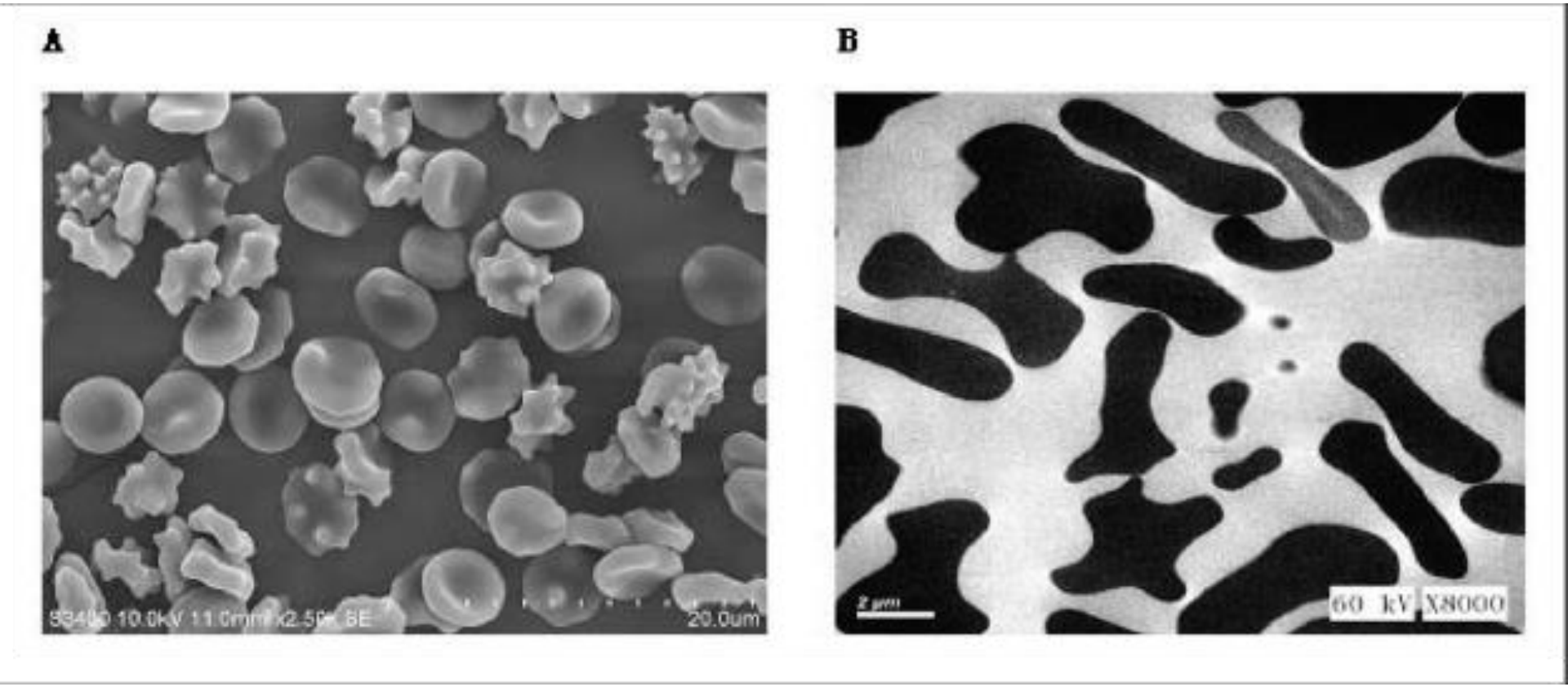
Ernst Ruska
1931

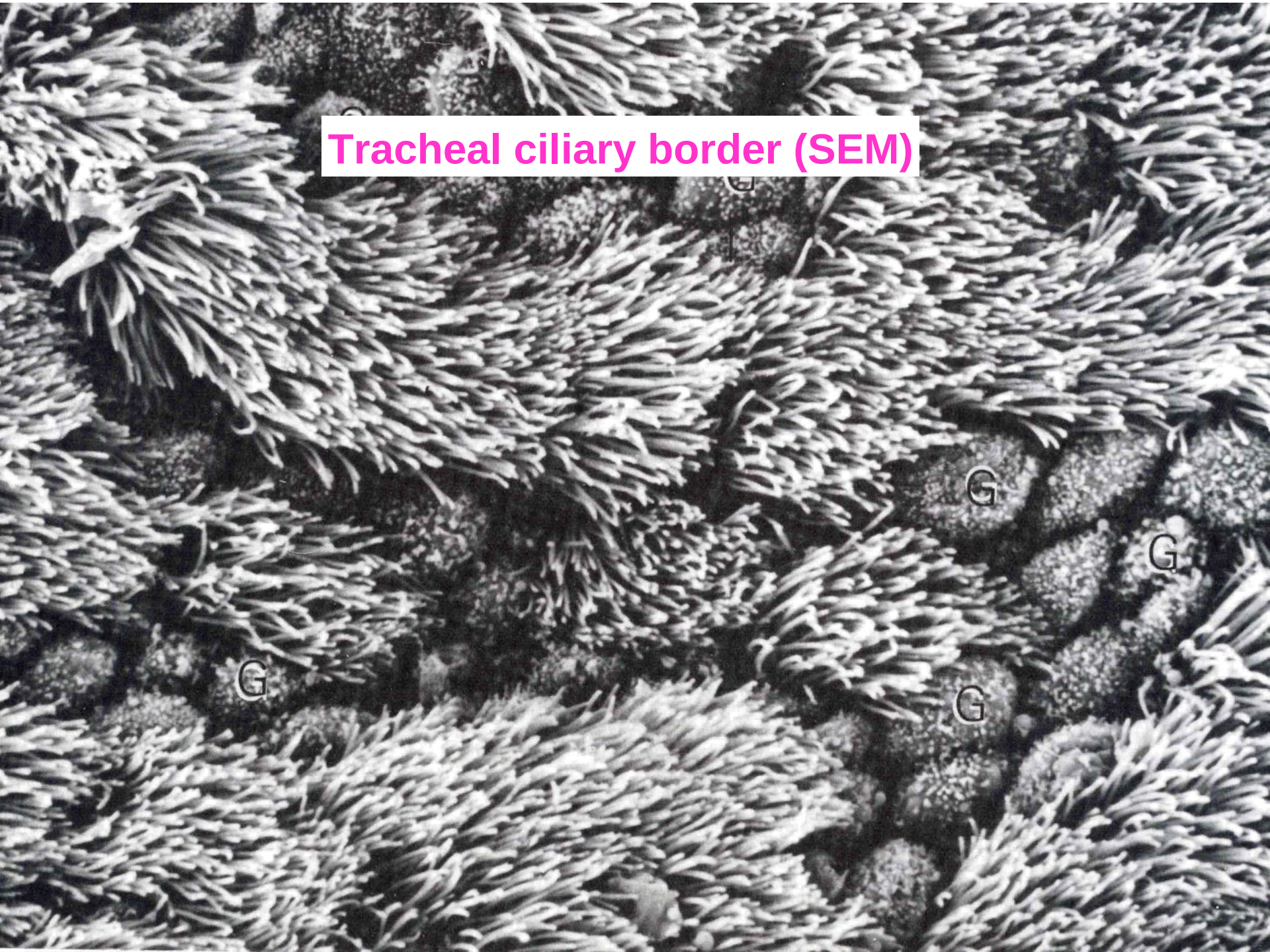


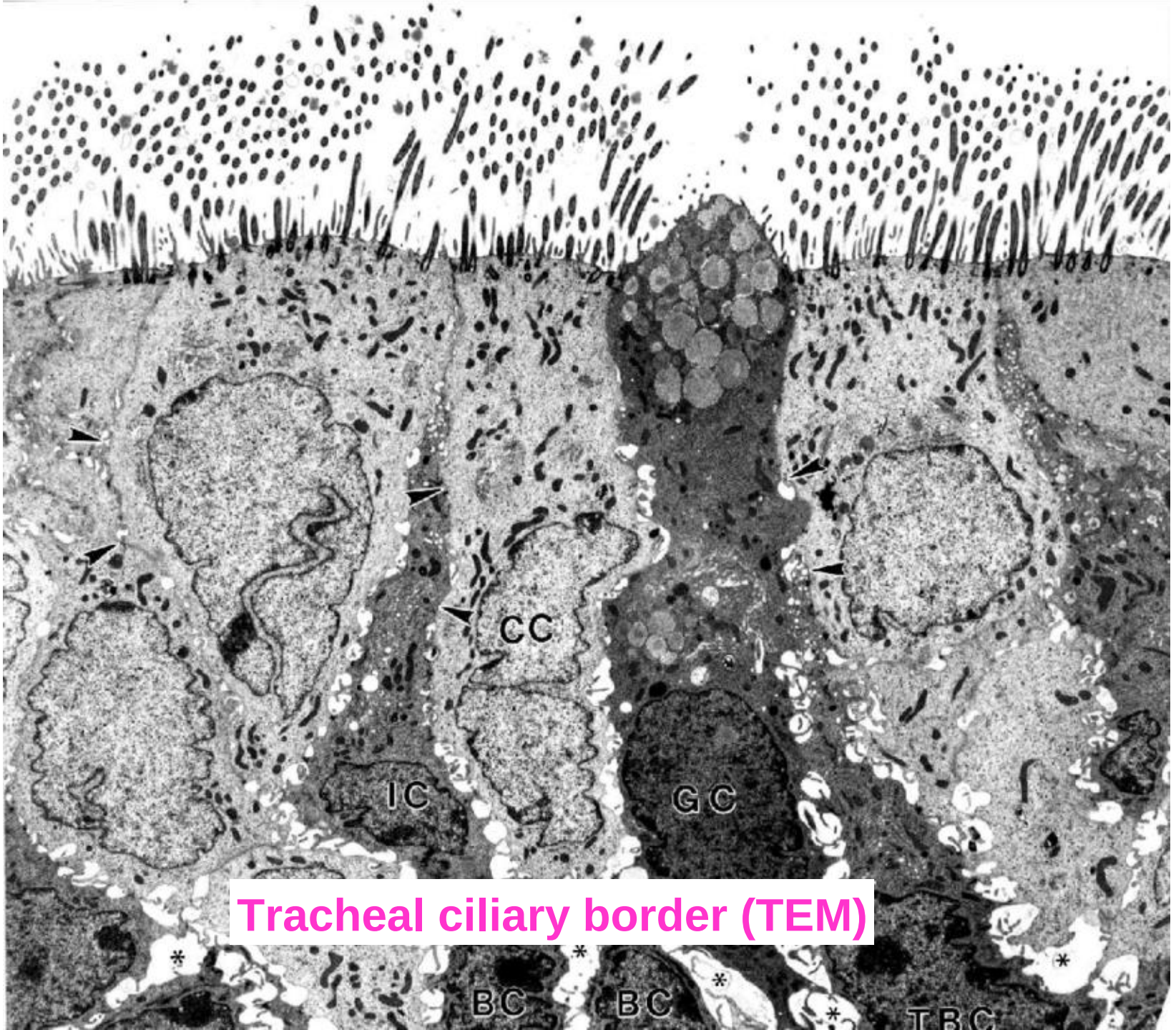
Scanning electron microscope (SEM)

SEM

TEM




Tracheal ciliary border (SEM)
G
G
G
G
G



Tracheal ciliary border (TEM)

HISTOCHEMISTRY

formation of

coloured reaction product

in situ

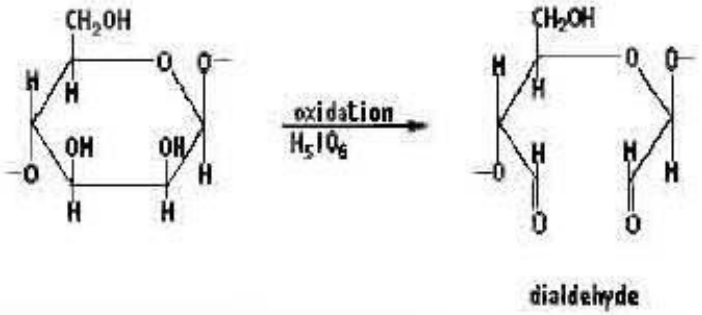
(reagent originally colourless)

Detection of
- elements (ions)
- nucleic acids
- lipids
- saccharides
- pigments
- proteins (aminoacids) – currently unusual, immunohistochemistry used instead

PAS reaction (periodic acid – Schiff)

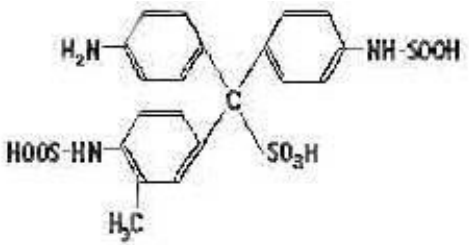
incompletely specific oxidative method detecting complex carbohydrates in cells and tissues

1 oxidation of free *vic* glycol groups by periodic acid (Malaprade reaction)

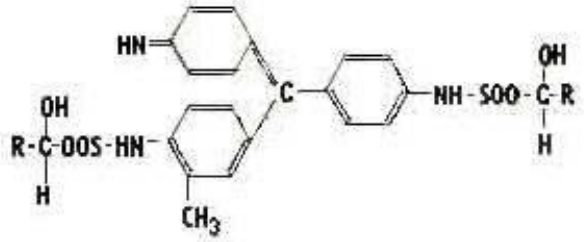


2 detection of just formed aldehyde groups by Schiff reagent

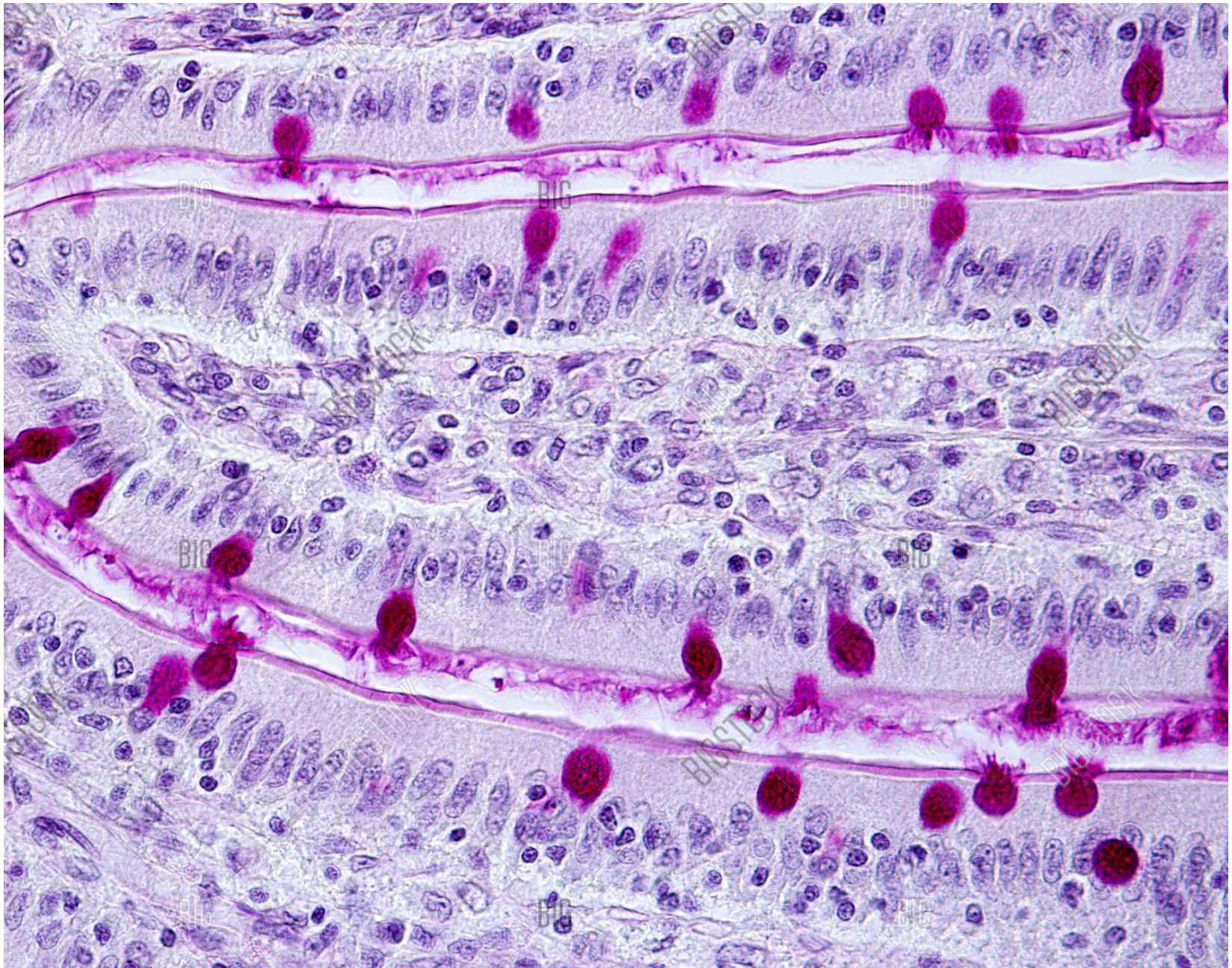
Schiff reagent – leukoform of basic fuchsin (colourless)



having reacted with aldehydes, a new magenta compound arises



PAS reaction **neutral mucins**



IMMUNOHISTOCHEMISTRY

1/ Bond of antigen and antibody

2/ Visualization of this complex